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Research Paper

Ibuprofen-enhanced biodegradation in solution and sewage sludge by a mineralizing microbial consortium. Shift in associated bacterial communities

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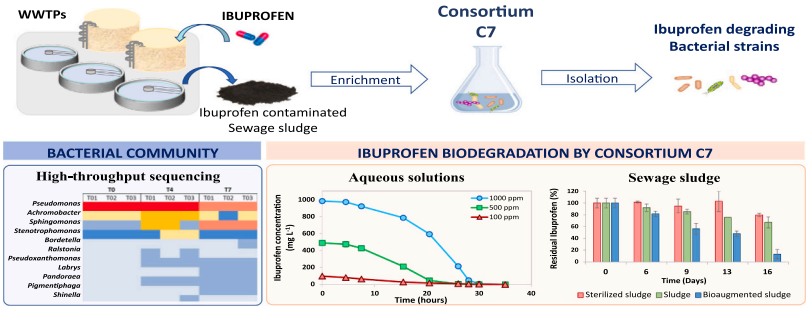
HIGHLIGHTS

- Sewage sludge microbial consortium to reduce ibuprofen (IBP) content by bioaugmentation.
- 100% IBP (1 mg L^{-1}) degraded in solution in 6 h.
- 90% IBP (10 mg kg^{-1}) degraded in sewage sludge in 16 days.
- *S. wittichii* the main potential bacterial specie responsible for IBP degradation.
- 8 isolated bacterial strains described for the first time as IBP-degraders.

GRAPHICAL ABSTRACT



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ABSTRACT

Ibuprofen (IBP) is a widely used drug of environmental concern as emerging contaminant due to its low elimination rates by wastewater treatment plants (WWTPs), leading to the contamination of the environment, where IBP is introduced mainly from wastewater discharge and sewage sludge used as fertilizer. This study describes the application of a consortium from sewage sludge and acclimated with ibuprofen (consortium C7) to accelerate its biodegradation both in solution and sewage sludge. 500 mg L^{-1} IBP was degraded in solution in 28 h, and 66% mineralized in 3 days. IBP adsorbed in sewage sludge (10 mg kg^{-1}) was removed after bioaugmentation with C7 up to 90% in 16 days, with a 5-fold increase in degradation rate. This is the first time that bioaugmentation with bacterial consortia or isolated bacterial strains have been used for IBP degradation in sewage sludge. The bacterial community of consortium C7 was significantly enriched in *Sphingomonas wittichii, Bordetella petrii, Pseudomonas stutzeri* and *Bosea genosp.* after IBP degradation, with a special increase in abundance of *S. wittichii*, probably the main potential bacterial specie responsible for IBP mineralization. Thirteen bacterial strains were isolated from C7 consortium. All of them degraded IBP in presence of glucose, especially *Labrys neptuniae*. Eight of these bacterial strains (*B. tritici, L. neptuniae, S. zoogloeoides, B. petrii, A. denitrificans, S. acidaminiphila, P. nitroreducens, C. flaccumfaciens*) had not been previously described as IBP-degraders. The bacterial community that makes up the indigenous consortium C7 appears to have a highly efficient biotic degradation potential to facilitate bioremediation of ibuprofen in contaminated effluents as well as in sewage sludge generated in WWTPs.

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1. Introduction

Ibuprofen (IBP) belong to the group of non-steroidal anti-inflammatory drugs (NSAIDs) used to treat pain and inflammation, and is the third most consumed drug in the world [45], with an annual output about 45,233 tons in 2022 [86]. Within Europe, IBP is considered by the Priority Substance Directive [22] and the EU commission [19] as candidate for the relatively short priority substance list under the Water Framework Directive [81]. Austin et al. [8] used the model ePIE (exposure to Pharmaceuticals in the Environment) to predict IBP concentrations in water bodies of several European countries, observing that its per capita consumption varied by nearly a factor of 10, with Spain having the highest usage (14,666 mg) and France the lowest (1809 mg). High amounts of IBP and its metabolites are excreted by humans and collected at the wastewater treatment plants (WWTPs). Although different treatments are applied, IBP elimination is not complete, remaining in part in the effluent treated waters and in part adsorbed on sewage sludge due to the high hydrophobic character of IBP molecule. WWTPs effluents are discharged in surface waters or used for soil irrigation, and IBP can be utilized by plants or aquatic organisms. Also the direct sewage sludge discharge on soils as fertilizers is a way to enter IBP and other organic contaminants into soils [36], and also into groundwater by leaching due to soil irrigation. Due to its for over consumption and inappropriate disposal, the presence of IBP has been detected in different environmental compartments (wastewater, surface water, groundwater, sludge, soils, sediments) [89]. In the era of the COVID-19 pandemic, drugs belonging to NSAIDs were detected in the world's water systems at concentrations ranging from a few nanograms to hundreds of micrograms per litre, being the most frequently detected diclofenac, IBP, naproxen, acetaminophen and ketoprofen [84]. In Spain, IBP (73–126 ng/L) was detected in urban groundwater in the urbanized areas of the metropolitan district of Barcelona [35]. In sewage sludge, the higher concentrations have been described for antibiotics and NSAIDS, being IBP one of the most frequently detected [44]. Although the concentrations of IBP and its metabolites which reach the environment are not excessively high, their continuous introduction in the different ecosystems results in long-term exposure. Therefore, IBP is considered as an emerging pollutant, since it is an artificial substance introduced in the environment from anthropogenic uses and activities, causing damage to ecosystem [15]. Praveenkumarreddy et al. [61] calculated Hazard Quotient (HQ) for various aquatic organisms for the effluents of WWTPs and river water, observing that IBP was toxic to *Vibrio fischeri*, *Daphnia magna* and algae. Also Thalla and Vannarath [76] have shown a high ecotoxicological risk of IBP contained in urban wastewater for fish and algae; Ajibola et al. [4] observed toxic effects of wastewater from treatment plants located near hospitals for fishes, and Parolini [60] detected IBP in the tissue of freshwater invertebrates. The accumulation of IBP and its metabolites in plant tissues have been also observed, with the potential transfer to other organisms, including humans, although their concentrations were not able to induce phyto-toxic effects [20,82].

Considering the wide use of IBP worldwide and the ecological risks involved, its removal from water and sewage sludge in WWTPs should be of priority interest since this is the unique opportunity to remove IBP before its release and dispersion in the environment. Physical, chemical and biological methods have been proposed for IBP removal from wastewater and sludge [15]. Among the physical methods, the adsorption on different matrices/filters is the most used: activated carbon (powder and granular), activated biochar, graphene, clay materials, polymer adsorbents, etc. [73]. IBP removal by chemical methods include advanced oxidation processes (AOPs) such as Fenton processes, TiO_2 photocatalysis, plasma oxidation, ozonation, or electrochemical oxidation processes [21,28]. However, research in advanced oxidation processes is still at an early stage, and IBP metabolites produced in these processes are usually more toxic than the parent compound [23]. Biological methods proposed to remove organic contaminants in general include the use of microorganisms (bacteria, fungus and algae) to degrade specific pollutants [65]. The use of bioremediation methods provides advantages because it is a cost-effective and environmentally friendly technique that offers the possibility to destroy or produce harmless organic contaminants using natural biological activity, and usually is well accepted by the general public [51].

In the case of IBP, studies on fungal and algal strains were limited [11], but several bacterial strains were known to degrade it [16,46,72,87], although only a few of them were able to mineralize IBP, belonging most of them to the family *Sphingomonadaceae*, such as *Sphingomonas* sp. Ibu-2 [53,54], *Sphingobium yanoikuyae* [10], *Rhizorhabdus wittichii* MPO218 [6], or *Sphingopyxis granuli* RW412 [3] Most of the studies have been conducted at a laboratory scale, not being applied in the environment yet.

In relation to microbial consortia, to date very few studies have been carried out for IBP degradation, and most of them used directly activated sludge from WWTPs without any enrichment with IBP [13,25,26,33,63,64,90]. Chen et al. [14] used a consortium from WWTP activated sludge but after six enrichment cultures with IBP. Other microbial consortia different from activated sludge were also used such as the microbial community from river sediments affected by a WWTP [71], or from surface water enriched with IBP [27]. As far as we know, there are no more studies using enriched microbial consortia for IBP biodegradation, but there are some studies using bacterial consortia prepared with defined bacterial strains. Marchlewicz et al. [47] used a consortia prepared with *Bacillus thuringiensis* B1(2015b) + *Pseudomonas moorei* KB4, and Wittich et al. [83] have described a consortia formed by *Pseudomonas citronellolis* RW422, RW423, RW424 which was able to mineralize IBP.

With regard to sewage sludge, so far only a few studies have investigated the removal of organic contaminants in general from sludge through bioaugmentation using bacteria as well as fungi or yeasts [29,39,75]. In the case of pharmaceutical products, studies on sludge treatment to achieve its decontamination have been much more scarce. Rodríguez-Rodríguez et al. [70] y Vilaplana et al. [79] observed that bioaugmentation using the white-rot fungus *Trametes versicolor* resulted in the degradation of several emerging pollutants (certain pharmaceutical products, polybrominated flame retardants, UV filters). Aydin [9] also used this fungus to biodegrade antibiotics in the sludge from a pharmaceutical industry. But, as far as we know, only Liu et al. [41] used bacterial consortia for gentamicin biodegradation in synthetic medium and raw gentamicin sewage.

Based on these knowledge gaps highlighted, in this work we have performed enrichment cultures from sewage sludge samples, a reservoir of diverse microbes with a high degradation potential for pharmaceuticals. The objectives of the current study were: (i) to obtain aerobic bacterial consortium and isolated bacterial strains capable of reaching the degradation of IBP; (ii) to assess the degradation efficiency of IBP both in solution and in sewage sludge under different conditions; (iii) to investigate bacterial community changes in the enriched ibuprofen-degrading biomass using high-throughput sequencing techniques to identify the main bacterial groups potentially involved in IBP bioremoval.

2. Materials and methods

2.1. Chemical, materials and culture media

The analytical standards of IBP ($C_{13}H_{18}O_2$, purity >98%, CAS: 15687–27–1, molecular weight: 206.28 g mol^{-1}) and IBP sodium salt ($C_{13}H_{17}O_2Na$, purity >98%, CAS: 31121–93–4, molecular weight: 228.26 g mol^{-1}) were purchased from Sigma-Aldrich (Madrid, Spain). The fresh sewage sludge was collected from a wastewater treatment plant (WWTP) in Seville (Southwest Spain). The most relevant physicochemical properties of the sludge used were described by Vargas-Ordóñez et al. [78], with an organic matter content of 48% and pH 8.25.

The mineral salt medium (MSM) contains the following components (per liter of deionized water): 0.5 g of KH_2PO_4, 0.5 g of K_2HPO_4, 0.01 g of NaCl, 0.2 g of $MgCl_2{\cdot}6\ H_2O$, 0.02 g of $CaCl_2$, 1 g of $(NH_4)SO_4$, 0.339 mg of $MnSO_4$, 0.428 mg of $ZnSO_4$, 0.347 mg of $(NH_4)_6Mo_7O_{24}{\cdot}4\ H_2O$, 0.4 mg of $CoCl_2{\cdot}6\ H_2O$, 5 mg of $FeSO_4{\cdot}7\ H_2O$, 0.2 mg of $CuSO_4{\cdot}5\ H_2O$, and 10 mg of EDTA. The pH was adjusted to 7.3 ± 0.1 with 1 M NaOH. Luria-Bertani (LB) broth contains (per liter of deionized water): 10 g tryptone, 5 g yeast extract, and 10 g NaCl (BD Difco™, Fisher Scientific). For LB-agar and MSM-agar media, 20 g L^{-1} of bacto agar or Difco®Agar Noble (BD Biosciences) were added to LB and MSM media, respectively. All culture media were autoclaved at 121 °C for 20 min.

2.2. Enrichment culture

The IBP-degrader consortium was obtained through an enrichment culture adding 5 g of activated sludge in 45 mL of MSM medium supplemented with IBP (an amount of solid IBP corresponding to 200 mg L^{-1}) as sole carbon source. The culture was incubated at 30 °C on a rotatory shaker at 180 rpm. Weekly, 5 mL of the culture was transferred to another flask with 45 mL of fresh MSM medium spiked with IBP (200 mg L^{-1}) and incubated again in the same conditions. After seven enrichment transfers, the consortium C7 was obtained. An aliquot of the resulting consortium was stored in Eppendorf microtubes containing 20% of glycerol (v/v), and kept at − 80°C.

2.3. Acclimatization and biodegradation assay with IBP-degrader consortium

To achieve a faster biodegradation process, Consortium C7 was subjected to an acclimatization process. A pre-inoculum was prepared cultivating the consortium C7 in LB medium supplemented with IBP sodium salt (500 mg L^{-1}) at 30 °C on a shaker at 180 rpm. After 24 h, cells were harvested through centrifugation (7000 rpm, 10 min) and the resultant pellet obtained was washed twice in MSM medium. Then, the acclimatized consortium was inoculated in triplicated in flasks with 50 mL of MSM supplemented with IBP sodium salt (500 mg L^{-1}) as sole carbon and energy source at an initial optical density ($OD_{600\ nm}$) of 0.1 and incubated for 7 days (30 °C, 180 rpm). Non-inoculated flasks were incubated in parallel as abiotic control. At different incubation times, 1 mL samples were taken from the flasks to determine IBP residues remaining in solution. Samples were subjected to three cycles of freezing and thawing in order to break the cell walls, ensuring the recovery of IBP accumulated in the microbial biomass. Afterwards, samples were centrifuged at 11,000 rpm for 2 min and IBP concentration measured in the supernatant at different times by a HPLC analyser coupled to an UV–vis detector (LC-2010AHT, Shimadzu), with a C-18 column (4 ×150 mm) and methanol:water (80:20) as mobile phase, acidified at pH 3 with orthophosphoric acid (1%), at a flow rate of 1.2 mL min^{-1}. The detection wavelength was 210 nm and the injection volume was 25 μL, and the detection limit was 0.05 mg L^{-1} of IBP. Bacterial growth was determined by optical density ($OD_{600\ nm}$) on a VWR UV-3100 spectrophotometer and by colony-forming units (CFUs) of serial dilutions on LB-agar medium plates.

After acclimatization process, IBP biodegradation assays in solution using C7 consortium were performed in triplicate. Cells from the acclimatized culture were harvested by centrifugation (7000 rpm, 10 min) after 7 days, when no IBP was detected in the culture medium, and inoculated at an initial $OD_{600\ nm}$ of 0.1 into new flasks with 20 mL of fresh MSM medium and different initial concentrations of IBP (1, 10, 100, 500 and 1000 mg L^{-1}) as sole carbon and energy source. These solutions were incubated on a temperature-controlled rotary shaker at 180 rpm and 30 ± 1 °C and protected from light, and at pH 7. The influence of other temperatures (20 °C and 40 °C) and pH (6 and 8) conditions on IBP degradation in solution with C7 consortium was carried out at the initial concentration of 500 mg L^{-1}. IBP concentration and bacterial growth were monitored at different times as previously described.

The possibility of IBP mineralization in solution by inoculating C7 consortium was studied using an OxiTop® BOD measuring system (OxiTop® IS 6 kit bottles) situated in a climate exposure test cabinet (30 ± 1 °C) (WTW GmbH, Weilheim, Germany). This equipment is a manometric respirometer in a closed system. The produced CO_2 due to the microorganism's respiratory process is adsorbed by soda-lime granules contained in the measuring heads of the OxiTop® bottles. The resulting pressure decrease shows the corresponding oxygen consumption, which was automatically calculated by the system. The OxiTop® bottles (500 mL) contained 97 mL of 500 mg L^{-1} Na-IBP as sole carbon and energy source in MSM solution, and were inoculated with the acclimatized consortium C7 at an initial optical density ($OD_{600\ nm}$) of 0.1 in triplicate and were left to incubate for 5 days. Blank control bottles without inoculation were also run.

2.4. Ecotoxicity bioassays

At different times in biodegradation process in solution using C7 consortium, the inhibitory effect of IBP and/or possible metabolites produced was studied through ecotoxicity assays based on the light emission of freeze-dried luminescent bacteria *Aliivibrio fischeri*, according to ISO 11348–3 [32]. The solutions were filtered to remove the remaining particulate matter and diluted with NaCl (2%) at 50%, 25%, 12.5% and 6.25% (v/v). A Microtox model 500 Analyser (Modern Water Inc., USA) was used to measure the decrease in luminescence after 15-min contact and compared with the control. EC_{50} value (IBP concentration (% v/v) having a toxic effect on 50% of the bacterial population) was calculated and expressed in Toxic Units (TU): $TU = 100/EC_{50}$ [43].

2.5. Total DNA extraction and bacterial community analysis of C7 consortium

To determine the bacterial community enriched in the acclimatization process and to identify potentially taxonomic groups involved in the IBP-degradation, total DNA was extracted in triplicate from 1 mL of the acclimatization culture at 4 and 7 days, as well as from the pre-inoculum (considered as time 0 days) using the G-spin™ Total DNA Extraction Kit (iNtRON Biotechnology, Inc). The quality and concentration of DNA were analysed by NanoDrop 2000 (Thermo Fisher Scientific). All DNA samples were submitted for high-throughput 16S rRNA gene amplicon sequencing to the Bioinformatics and Computational Biology Service at the Institute of Biomedicine of Seville (IBIS). The V3-V4 variable regions of the 16S rRNA gene were amplified by PCR using universal primers 341 F (5'-CCTACGGGNGGCWGCAG-3') and 805 R (5'-GACTACHVGGGTATCTAATCC-3'). Amplicons were sequenced on the Illumina MiSeq platform using 600-cycle MiSeq Reagent Kit v3 (Illumina, San Diego, CA, USA). All sequences files were deposited at the National Center for Biotechnology Information (NCBI) Sequences Read Archive (SRA – http://www.ncbi.nlm.nih.gov/sra) under BioProject ID: PRJNA1002128.

2.6. Bioinformatic and statistical data analysis

Sequencing data of 16S rRNA were processed using Mothur software (v.1.43.0). Silva NR database (v.138) was employed to align the representative sequences of each operational taxonomic units (OTUs) [62], whereas Greengenes database (v.13_8_99) was used for classification and taxonomic purposes. OTUs were classified at 97% sequence similarity to detect subgenera. Principal coordinates analysis (PCoA), hierarchical cluster and non-metric multidimensional scaling (NMDS) analysis of the bacterial community of the consortium C7 based on Bray-Curtis distances (at the OTU, genus and specie level respectively) and the permutational multivariate analysis of variance (PERMANOVA) from OTU data were determined using PAST software version 4.13.

Alpha diversity parameters (Chao1, Shannon index and Simpson's evenness) were also quantified using PAST software. Mean significant differences were detected by One-way analysis of variance (ANOVA) at a significance level of 0.05 using the SPSS statistical software package version 25 (IBM Corporation, New York, USA).

2.7. Isolation and identification of bacterial strains from C7 consortium

To isolate the IBP-degrading bacterial strains, 100 μL of the consortium C7 after acclimatization were plated on both MSM-agar medium as LB-agar medium in presence of IBP (200 mg L^{-1}) and incubated at 30 °C for 48 h. Thirteen bacterial strains were isolated according to colony morphology (size, colour, edge and elevation) and growth capacity on MSM-agar medium with IBP as sole carbon source. For their identification, total DNA was extracted from 1 mL of pure LB cultures using G-spin™ Total DNA Extraction Kit (iNtRON Biotechnology, Inc). These bacterial strains were identified by sequencing the 1465 bp-long 16S rRNA gene amplification products obtained by PCR using the universal oligonucleotide primers 27 F (5-AGAGTTTGATCCTGGCTCAG-3) and 1492 R (5-GGTTACCTTGTTACGACTT-3). Sanger sequencing of the amplified products was performed at the STABVIDA laboratory (Caparica, Portugal). In addition, primers 518 F (5-CCAGCAGCCGCGGTAATACG-3) and 800 R (5-TACCAGGGTATCTAATCC-3) were used. The 16S rRNA sequences of isolates were submitted to the GenBank database in NCBI under different accession numbers.

2.8. Biodegradation assays in solution with isolated IBP-degrading bacteria

All bacterial strains isolated from the consortium C7 were inoculated at 10^8 CFU mL^{-1} under aerobic conditions in triplicated in flasks with 20 mL of MSM medium spiked with IBP (10 mg L^{-1}) and cultivated at 30 °C and 180 rpm for 14 days in presence of glucose (3 g L^{-1}). Non-inoculated sterile controls were also prepared to observe any abiotic degradation. IBP concentration in solution was determined by HPLC analysis as described in Section 2.3.

2.9. IBP biodegradation experiments in sewage sludge slurry

To determine the capacity of the consortium C7 to improve IBP removal rates in sewage sludge, bioaugmentation assays were performed, but previously adsorption-desorption experiments of IBP on sewage sludge were carried out. For that purpose, IBP batch adsorption experiments in sewage sludge were carried out in triplicate in Corex centrifuge tubes by mixing 0.1 g of sludge with 10 mL of 0.01 M $Ca(NO_3)_2$ solution containing various concentrations (0, 3, 5 and 10 mg L^{-1}) of IBP. The samples were shaken for 72 h at 20 ± 1 °C (previous kinetic studies showed that adsorption equilibrium was reached in less than 24 h). IBP adsorbed was calculated from the difference between its concentrations in the supernatant before and after equilibrium, and adsorption isotherms were obtained by plotting the amount of IBP adsorbed (C_s, μmol kg^{-1}) versus the respective concentrations in equilibrium (C_e, μmol L^{-1}). Sorption isotherm was fitted to the linear equation $C_s = K_d \times C_e$, where K_d is the linear sorption constant. Desorption experiments were performed after adsorption equilibrium was reached by removing 5 mL of the supernatant (for initial IBP concentrations of 3, 5 and 10 mg L^{-1}) after centrifugation and replacing it by 5 mL of 0.01 M $Ca(NO_3)_2$ solution, allowing equilibration for an additional 24 h, and finally operating as in the adsorption experiments. Three consecutive desorption steps were carried out.

IBP biodegradation experiments in sewage sludge were carried out in Corex glass centrifuge tubes containing 1 g of sludge which was spiked with 1 mL of 10 mg L^{-1} IBP solution prepared in methanol to obtain an initial IBP concentration of 10 mg kg^{-1}. After solvent evaporation, 5 mL of MSM medium were added to each tube, which were also inoculated to obtain 10^8 CFU mL^{-1} of consortium C7. Non-inoculated tubes were run in parallel as a control of the IBP dissipation in sewage sludge in order to observe any biodegradative effect of the sludge endogenous microbial population. For abiotic controls, sludge was sterilized by autoclaving at 121 °C for 40 min, and sodium azide was added in the MSM medium at an initial concentration of 200 mg L^{-1} to avoid the biological activity. All microcosms were incubated at 30 °C on a rotatory shaker at 150 rpm for 16 days. The IBP concentration was monitored at 0, 6, 9, 13 and 16 days. The centrifuge tubes were freeze-thawed three times to break the cells walls and then the samples were centrifuged at 7000 rpm for 20 min. The supernatant was discarded after verifying by HPLC the absence of IBP in the liquid phase as described in Section 2.3. Then, an exhaustive IBP-extraction from the solid phase was performed by adding 5 mL of acetonitrile acidified with 1% acetic acid and vortexing for 1 min. Samples were supplemented with 2 g of a QuEChERS extraction salts mixture (Agilent Technologies, Santa Clara, CA, USA), vortexed for 1 min, and centrifuged at 7000 rpm for 10 min. The supernatants were transferred to new vials and 200 mg of PSA (Primary Secondary Amine, Agilent Technologies, Santa Clara, CA, USA) were added and manually shaked. After 24 h decantation, 1 mL aliquots were filtered through a 0.45 μm PTFE filters and analyzed by HPLC analyser coupled to an UV–vis detector (LC-2010AHT, Shimadzu), with a C-18 column (4 ×150 mm) and acetonitrile:water (50:50) as mobile phase (pH 3 with orthophosphoric acid), at a flow rate of 1.2 mL min^{-1}. The detection wavelength was 210 nm, the injection volume was 25 μL and the detection limit was 0.05 mg L^{-1} of IBP.

3. Results and discussion

3.1. Biodegradation of IBP in solution by the enriched consortium C7

After seven enrichment cultures an efficient and stable IBP-degrading consortium was successfully obtained. An acclimatization period of three days was necessary to begin IBP degradation and the growth of the consortium biomass with IBP as the sole carbon source, removing 100% of 500 mg L^{-1} in six days (Fig. 1), and increasing the growth of the consortium from an optical density $OD_{600\ nm}$ of 0.1–0.5. It indicated that C7 consortium is composed of a microbial population capable of degrading (and probably mineralizing) IBP, and, therefore, its potential application for its removal from water and sewage sludge. A similar experiment was carried out but using glucose as an additional carbon source to observe if the kinetic of IBP degradation could be reduced, but IBP degradation was completely inhibited, indicating that microorganism of the consortium preferentially select such easily degradable carbon source.

In order to reduce the time of IBP degradation by consortium C7, the rest of assays were carried out using cells from the acclimatized culture harvested after 7 days (when no IBP was detected), and no acclimatization period was observed, as shown in Fig. 2 where different IBP concentrations (1–1000 mg L^{-1}) were proved at pH 7 and 30 °C.

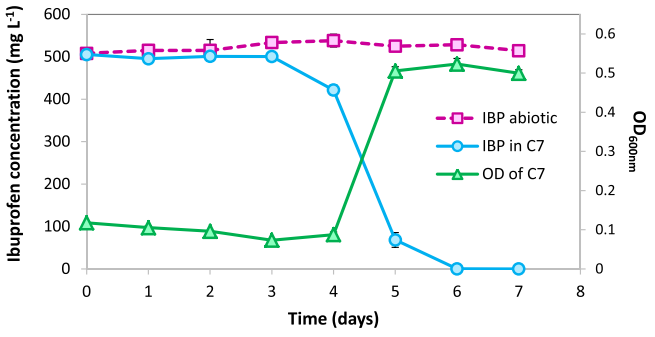

Fig. 1. Ibuprofen (500 mg L^{-1}) degradation efficiency and growth of the consortium C7 biomass with ibuprofen as the sole carbon and energy source. Error bars indicate standard deviation.

Biodegradation curves of the control samples remained stable throughout the experiment (data not shown) and the abiotic dissipation could be considered negligible. For all the concentrations tested, lag phases were not perceived and IBP concentration sharply decreased, reaching 100% degradation in less than 30, 28, 26, 24 and 6 h for the concentrations of 1000, 500, 100, 10 and 1 mg L^{-1}, respectively. The growth of the consortium ($OD_{600\ nm}$) drastically increased from 0.1 till 0.72 and 0.45 for 1000 and 500 mg L^{-1} IBP, respectively, and only till 0.15 for 100 mg L^{-1}. The increase was not observed for 10 and 1 mg L^{-1}. It indicated the ability of the consortium C7 to degrade a wide variety of IBP concentrations in solution in a very short time, and also that IBP, and probably its degradation products, are being used by microorganisms in C7 consortium as carbon and energy source. The influence of other conditions of temperature and pH on IBP degradation ability of C7 consortium is shown in Fig. 3. In the range of pH 6–8 (at a temperature of 30 °C) the rate and efficiency of biodegradation was not affected (Fig. 3B). On the contrary, the change in temperature in the range among 20 °C and 40 °C (at pH 7) affected significantly the IBP biodegradation by C7 consortium (Fig. 3A). The most favourable temperature was 30 °C, with 100% IBP degraded in 28 h, in comparison to the 70 h necessary to reach the complete IBP biodegradation at 20 °C, and the null capacity of C7 consortium for biodegradation at 40 °C.

The rapid IBP biodegradation shown by consortium C7 even at very high concentrations of this drug (up to 1000 mg L^{-1} in only 30 h) and the use of IBP as carbon source for C7 consortium growth (Fig. 1) seems to indicate the possible ability of C7 to mineralize IBP. The conditions of temperature and pH selected for mineralization experiment were the most favourable previously shown: pH 7 and 30 °C, and the results are shown in Fig. 4. About 66% IBP was mineralized in only 3 days since it was the percentage of CO_2 produced due to the respiratory process of microorganisms present in C7 consortium. Therefore, the use of IBP as the only carbon and energy source, generating biomass and CO_2, has been demonstrated.

Toxicity measurements using Microtox bioassays were carried out throughout the bioremediation process in solution of one of the highest IBP concentrations used. It was observed that the initial concentration of 500 mg L^{-1} in the non-inoculated microcosms at the beginning of the process presented acute toxicity (TU 8.87), which was slightly increased 20 h after inoculation (TU 9.70), in spite of presenting an IBP remaining concentration of only 68 mg L^{-1} (Fig. 2A). It probably indicates that some of the metabolites produced through this process are even more toxic than the parent contaminant. However, when IBP was completely degraded after 24 h, the solution presented no-toxicity (TU 0).

As previously mentioned, very few studies have been carried out using microbial consortia for IBP degradation. Jia et al. [33] and Quintelas et al. [64] used activated sludge from WWTPs and observed the complete degradation of 1 mg L^{-1} IBP after 36 h and 50 h, respectively, while the bacterial consortium C7 used in the present study took only 6 h to degrade the same IBP concentration. In general, the results obtained with consortium C7 in the present study are better than most of those previously mentioned in the Introduction section using different consortia. They only were similar to those of Ferrer-Polonio et al. [26] with a degradation of 2 mg L^{-1} IBP in only 5 h using activated sludge,

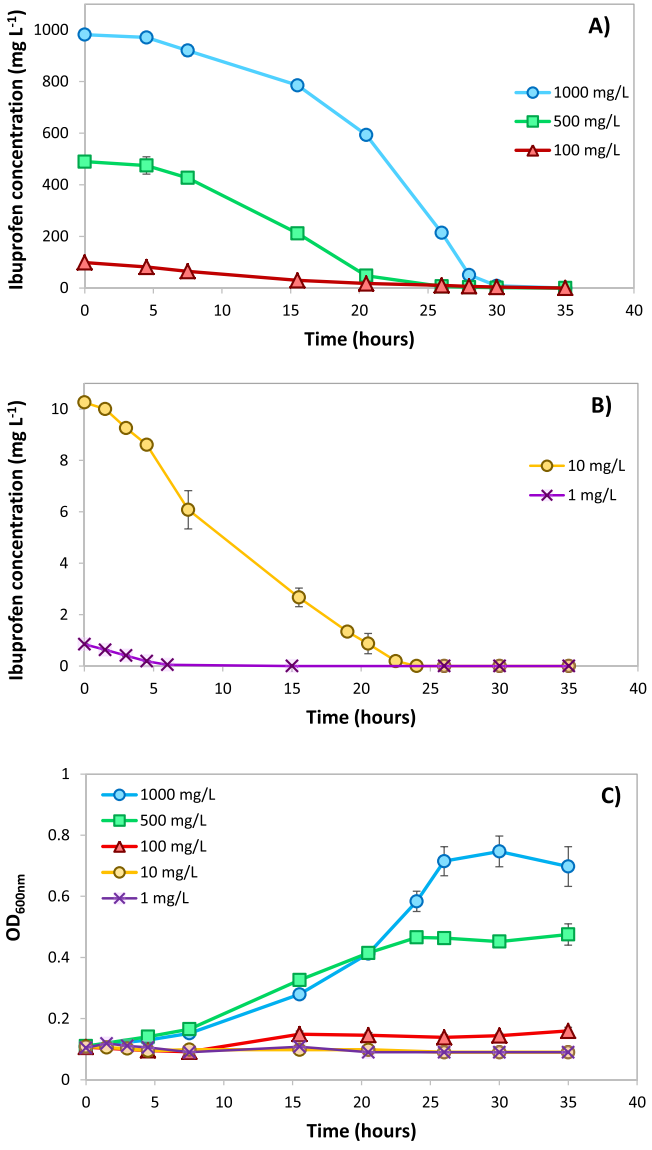

Fig. 2. Influence of different ibuprofen concentrations on degradation efficiency of consortium C7 (A and B) and growth of biomass (C) at pH 7 and 30 °C. Error bars indicate standard deviation.

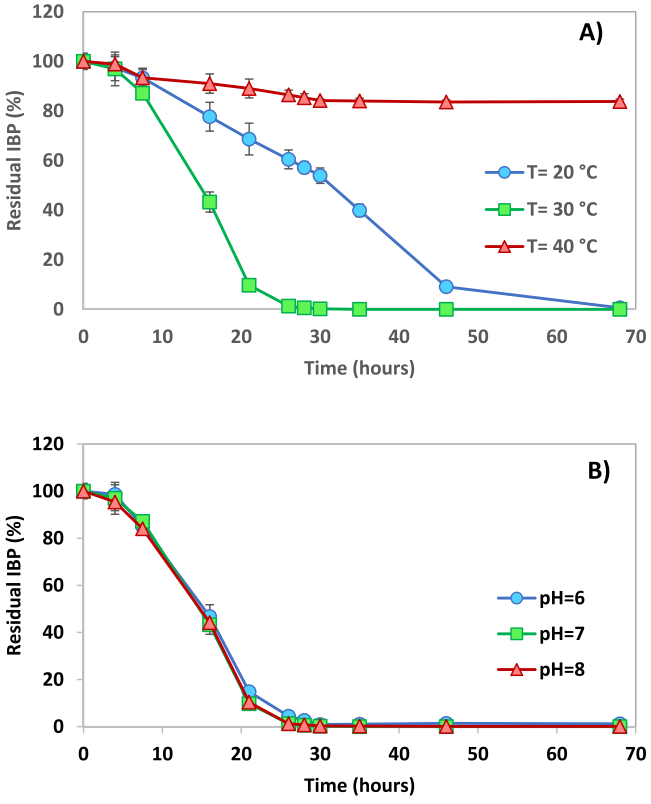

Fig. 3. Ibuprofen degradation profiles in solution by consortium C7 under different conditions of (A) temperature (at pH 7), and (B) pH (at 30 °C).

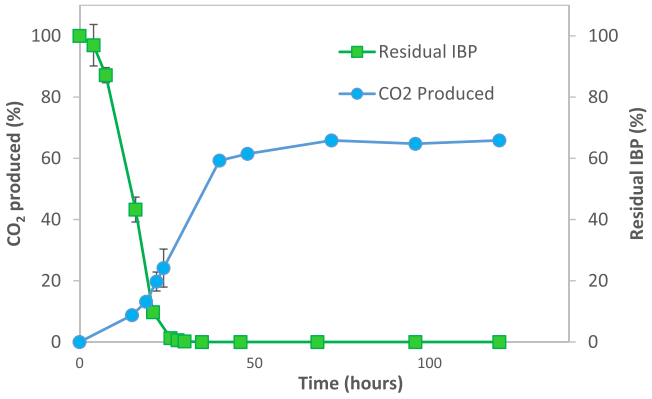

Fig. 4. Mineralization of Ibuprofen (500 mg L^{-1}) measured as the cumulated percentage of CO_2 produced by the respiratory process of C7 consortium, as related to the corresponding IBP biodegradation (30 °C, pH 7).

and those of Fortunato et al. [27], with 100 mg L^{-1} IBP degraded in 33 h. The results obtained by Chen et al. [14] using a bacterial consortium from activated sludge after enrichments with IBP were also similar to those obtained in the present study, with 50 mg L^{-1} IBP degraded in 12 h. With regard to bacterial consortia prepared with defined bacterial strains, the use of *Bacillus thuringiensis* B1(2015b) + *Pseudomonas moorei* KB4 [47] reached a degradation of 5 mg L^{-1} IBP in 24 h, similar also to the results obtained with consortium C7 in the present study. All these results indicated that the consortium C7 had a greater potential than most of the consortia published in literature for a real application in IBP degradation.

3.2. Biodegradation of IBP in sewage sludge by the enriched consortium C7

A great part of sewage sludge produced in WWTPs is valorised as amendment in agriculture, and according to Lachassagne et al. [37], IBP shows a high desorption potential from sludge and high mobility in soils, being found in amended soil leachates, with the consequent risk of groundwater contamination, and the negative effects for different ecosystems. Other studies have also demonstrated the low sorption affinity of IBP in soils [24,40]. Therefore, one of the objectives of the present study was to reduce IBP concentration also in sewage sludge samples.

Adsorption-desorption isotherms of IBP on the sewage sludge selected for its degradation studies were carried out (Fig. 5). IBP adsorption was well described by a linear equation and the sorption constant K_d obtained was 70.2 L kg^{-1}, similar to K_d values obtained by Abegglen et al. [1] and Urase and Kikuta [77] (< 75 L kg^{-1} and 80 L kg^{-1}, respectively), which indicated a weak sorption to the sludge. Yu et al. [88] and Hörsing et al. [30] also observed a weak sorption of IBP by sludge. In contrast to literature reports, in our case the percentage desorbed was very low, only about 12–15%, as it can be observed in Fig. 5 for initial IBP concentrations of 3, 5 and 10 mg L^{-1}. IBP exhibit a log Kow (octanol-water coefficient) of 3.97 which was supposed to lead to low affinity for the aqueous phase. Sewage sludge under study presented a high organic matter content (48%) and probably the hydrophobic adsorption of IBP on the sludge played a special role in this case, but it is clear that desorption ability based on Kow or pKa is not always reliable, as previously observed in other studies. Mejías et al. [48] observed that one of the pharmaceuticals detected at higher concentrations in digested sludge was IBP (up to 4105 ng g^{-1}), but also observed that its concentrations significantly differ between studies even up to three orders of magnitude. This is mainly related to sludge source, which depends on the different human habits, sampling regions or climate conditions.

The next step was to test if the consortium C7 was able to decrease the concentration of IBP adsorbed on sewage sludge. As previously

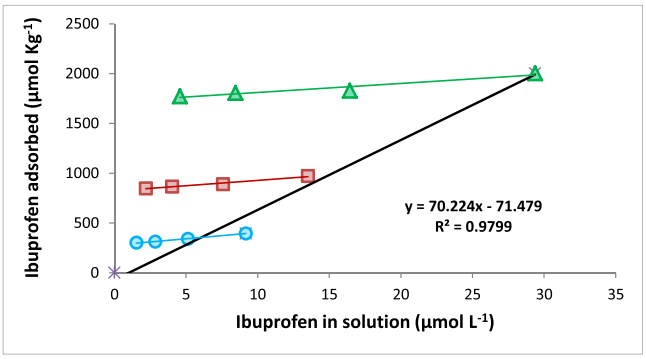

Fig. 5. Adsorption-desorption isotherms of Ibuprofen on sewage sludge using 0.01 M $Ca(NO_3)_2$ as desorbent. Desorption from initial IBP concentrations of 3, 5 and 10 mg L^{-1}.

mentioned, few studies are available at the moment to eliminate pharmaceuticals from sewage sludge by using bioaugmentation, and most of them used fungus [9,68,69]. As far as we know, only Liu et al. [41] studied the biodegradation of gentamicin by the bacterial consortia AMQD4, removing about 57% and 48% in unsterilized and sterilized sewage, respectively. Fig. 6 shows the percentages of residual IBP in the solid phase of the slurry. No IBP was detected in the liquid phase of the slurry under the different conditions tested, corroborating the previous results obtained of very low percentages of IBP desorbed from the sewage sludge selected for this study.

In general, the mechanisms involved in the removal of pharmaceuticals by microorganisms include biodegradation, bio-sorption, volatilization and hydrolysis [64], but several studies observed that IBP was not removed via volatilization and hydrolysis [88]. Therefore, the main mechanisms involved in the dissipation of IBP are biodegradation and biosorption on sewage sludge. Separation of these processes was achieved using sodium azide to inhibit the biotic activity, and this assay is called "Sterilised Sludge". After applying an exhaustive extraction method, 100% of IBP adsorbed on the sludge could be extracted after different incubation periods, and only after 16 days a lower percentage of IBP (80%) was extracted from the sludge, indicating that after longer periods a small part of IBP could remain as a bound residue.

When the sludge was not sterilised (assay called "Sludge"), a continuous but very small decrease of IBP remaining on sludge was observed as time increased, indicating that the endogenous microbiota of the sludge was capable of degrading IBP despite being strongly adsorbed, as previously commented. After 16 incubation days only about 30% IBP had been dissipated, with a great part corresponding to biosorption and the rest to biodegradation, since, as previously mentioned in "Sterilized Sludge", about 20% IBP remained bioadsorbed, and, therefore, only 10% can be assigned to biodegradation.

When the sludge was bioaugmented with consortium C7 ("Bioaugmented Sludge") a faster IBP degradation was observed, and after 16 days around 90% IBP had been removed from the sludge mainly due to biodegradation, in spite of the strong adsorption previously observed. It demonstrated the suitability of consortium C7 to biodegrade IBP not only in solution, but also adsorbed on sewage sludge, even as bound residue. As far as we know, no studies have been conducted to date on bioaugmentation using bacterial consortia or isolated bacteria for IBP degradation in sludge. Therefore, it is important to highlight the application significance and the potential environmental impact that this novel bioaugmentation technique using consortium C7 isolated from sewage sludge could have for its use in a real environment of a WWTP, in order to reduce the content of IBP residues both in wastewater and sludge.

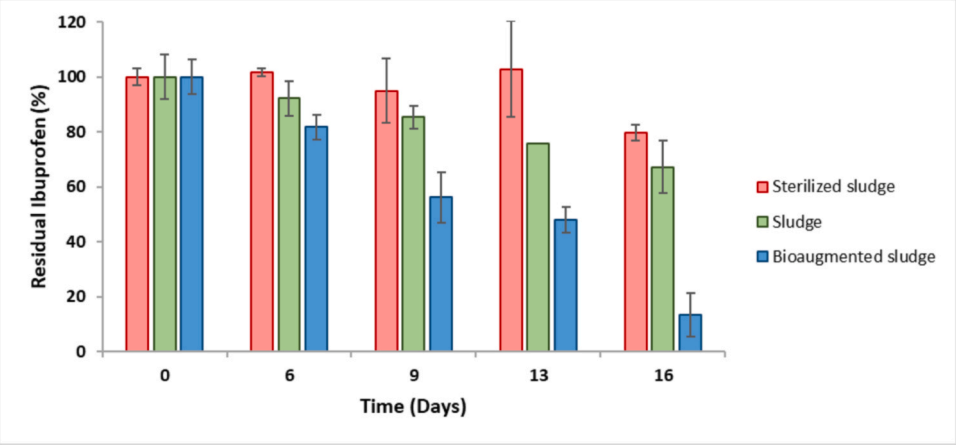

Fig. 6. Percentages of residual ibuprofen in sewage sludge (initial concentration 10 mg kg^{-1}) at different conditions: sludge and sterilized sludge (both non-inoculated with consortium C7) and sludge bioaugmented with consortium C7. Error bars indicate standard deviation.

3.3. Bacterial community structure and composition of the consortium C7

IBP-degrading consortium C7 was analysed by amplicon sequencing of the 16S rRNA gene during acclimatization process at different times (0, 4 and 7 days, Fig. 1) to determine changes in the bacterial community composition and to identify the main groups of microorganisms potentially involved in IBP degradation.

Alpha diversity indices (Chao1, Shannon and Simpson) of the bacterial community in the consortium C7 were analyzed at 0, 4 and 7 days to determine the effect of IBP on the diversity and richness of the bacterial community during the acclimatization process (Fig. S1 Supporting Information). In general, the values of these parameters increased significantly over time (ANOVA, $p < 0.05$). According to Chao1 index, the bacterial richness increased in the consortium C7 after four days of incubation with IBP as sole carbon and energy source. Similar tendencies were observed in Shannon diversity and Simpson indices, which showed significantly higher values at 7 days (ANOVA, $p < 0.05$). The tendencies of these parameters indicate that IBP positively affects both richness and diversity of the bacterial population during the acclimatization process. With regard to other previous studies, a positive effect on bacterial diversity was also observed in batch reactors and water biopurification systems treated with IBP, showing the resilience potential of bacterial communities exposed to this contaminant [2,34].

Hierarchical clustering analysis of the bacterial community in the consortium C7 grouped samples according to time (Fig. 7A), with 0-day samples clustering more closely with 4-day samples, when 80% of IBP still remained in the culture. Similarly, the first principal component (PC1) of the principal coordinate analysis (Fig. S2) accounts for 94% of statistical variability (PC1) separates 0-day and 4-day samples from 7-day samples. As expected, the permutational multivariate analysis of variance (PERMANOVA) from operational taxonomic units (OTUs) data showed significant differences between these groups ($p < 0.003$), indicating that IBP incubation time affected the bacterial composition of the consortium C7.

The bacterial community of the consortium C7 was integrated by 51 different OTUs grouped into the phyla *Proteobacteria* (92.7–99.9%), *Bacteroidetes* (0.01–7.2%), *Actinobacteria* (0–0.05%) and *Firmicutes* (0.01%) (Fig. S3). *Proteobacteria* have been previously described as the dominant phylum in activated sludge supplemented with high concentrations of IBP [18]. The relative abundance of the most dominant genera in the consortium C7 during the acclimatization process at 0, 4 and 7 days is shown in Fig. 7B. At the genus level, 29 different genera were detected, which accounted for 91.6–99.9% of the total OTUs annotated in all samples. *Pseudomonas* was the most predominant genus at the different times followed by *Achromobacter*, *Sphingomonas* and *Stenotrophomonas*. Despite the existence of IBP-degrading bacterial strains belonging to the genus *Pseudomonas* [14,71,83], the relative abundance of this genus was drastically decreased in the consortium C7 during the acclimatization process (Fig. 7B). The genera *Achromobacter* and *Stenotrophomonas* also showed lower relative abundance values after 7 days of incubation with IBP. In contrast, a 42.5% increase in the relative abundance of *Sphingomonas* occurred during acclimatization process (Fig. 7B). Therefore, the genus *Sphingomonas* could be potentially involved in the disappearance of IBP from the culture medium. According to this result, only few studies reported a correlation between the *Sphingomonas* genus and IBP-degradation. Murdoch and Hay [53] isolated the bacterial strain *Sphingomonas* sp. Ibu-2 from activated sludge, which was able to use IBP as sole carbon and energy source. Moreover, Huang et al. [31] suggested that the detection of sequences identical to *Sphingomonas sp*. could be in charge of IBP biodegradation in activated sewage sludge. In a previous study, *Sphingomonas*-related OTU was proposed as a potential biomarker involved in IBP-degradation in biopurification systems [2].

Increases in the relative abundance of *Pseudoxanthomonas*, *Labrys*, *Pandoraea* and *Pigmentaphaga* were also observed (Fig. 7C), thus suggesting the possible involvement of these genera in IBP dissipation. In fact, bacterial species belonging to these genera have been linked to the degradation of several non-steroidal anti-inflammatory drugs [42,50,80].

In order to elucidate the specific taxa potentially involved in IBP degradation, the different bacterial species comprising the consortium C7 were analysed during the acclimatization process (Fig. 8). Fifteen bacterial species were detected in the consortium C7. When NMDS ordination was constrained with data of the detected bacterial species (Fig. 8A), *Sphingomonas wittichii*, *Bordetella petrii*, *Pseudomonas stutzeri* and *Bosea genosp*. showed more influence at 7 days when no IBP was detected in the culture medium, indicating that these species could be potential IBP-degraders. Previously, these bacterial species have been related with an effective removal of several recalcitrant compounds. *Bordetella petrii* was identified in the bacterial community established in an immobilized cell biofilter with a high effectiveness to depurate diclofenac from wastewater [55]. On the other hand, *Pseudomonas stutzeri* was detected in biopurification systems exposed to IBP and considered an interesting biomarker for studying IBP degradation [2]. The bacterial strain *P. stutzeri* CSW02 has been also described as an efficient paracetamol degrader [78]. Recently, Aulestia et al. [7] reported the bacterial strain *Sphingomonas wittichii* MPO218 isolated from activated sludge with the capacity to growth with IBP as a sole carbon and energy source. In the current study, although *Pseudomonas nitroreducens* was the most dominant bacterial specie in the consortium C7 at

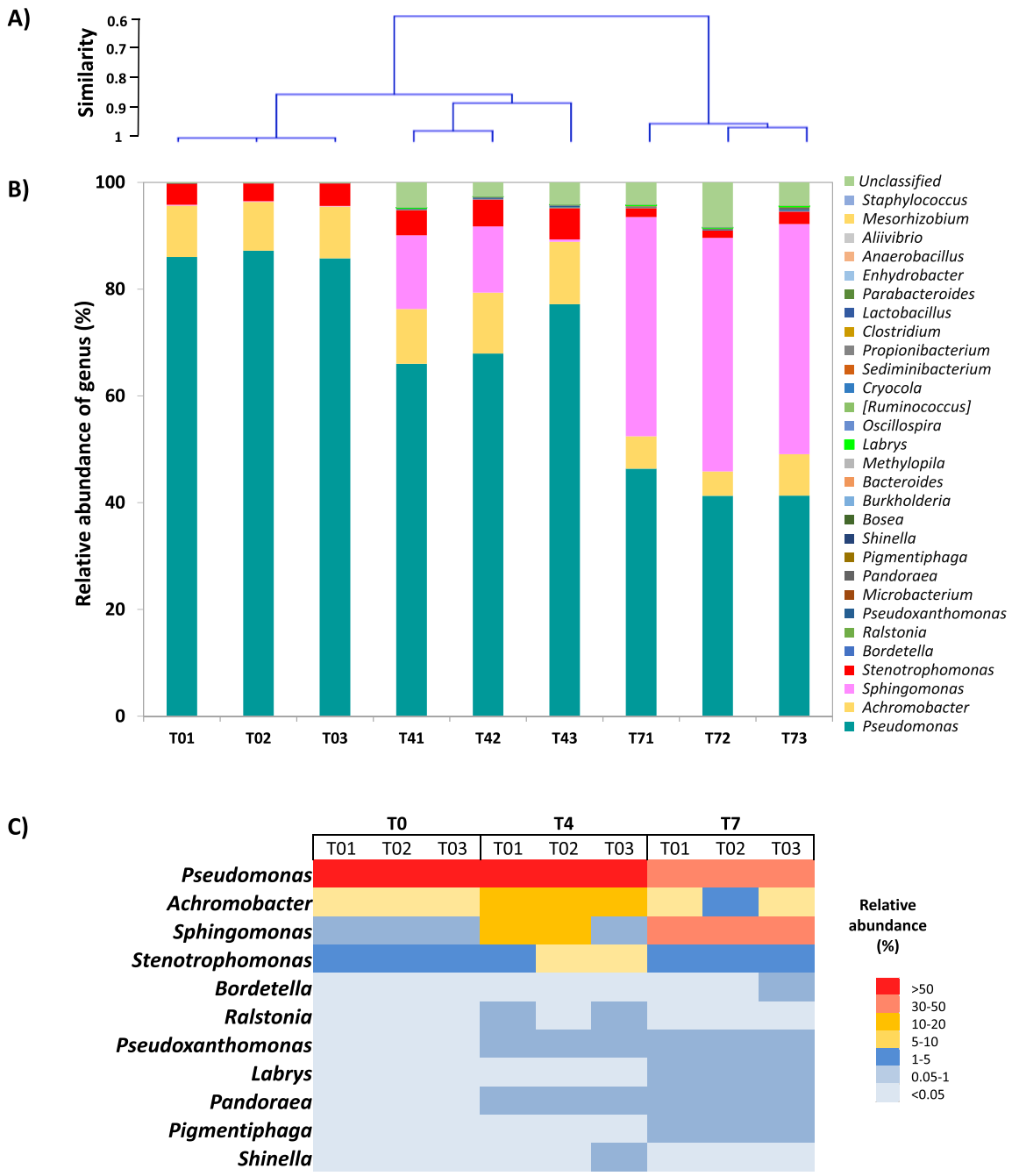

Fig. 7. Hierarchical cluster analysis of the microbial community of the consortium C7 based on Bray-Curtis distances (A). Relative abundance of the most dominant genera in the consortium C7 during the acclimatization process at 0, 4 and 7 days (B). Heatmap of the most abundant genera (relative abundance > 0.05% in any sample) in the consortium C7 at different times.

initial time (Fig. 8B), a strong enrichment of *S. wittichii* was observed after 7 days, which relative abundance increased from 0-0.03% to 40.8–43.4%. Thus, these results highlight *S. wittichii* as the main bacterial specie in the consortium C7 responsible for IBP degradation. This is reinforced by the fact that IBP mineralization by C7 consortium has been observed, and the conclusion reached by various researchers that the complete mineralization of IBP is limited to the family of the *Sphingomonadaceae*, the same as *S. wittichii*, when present as sole carbon and energy source. IBP exposure also caused an initial positive effect on *Stenotrophomonas acidaminiphila*, increasing its relative abundance to 5.3% after 4 days of incubation (Fig. 8B), and suggesting the possible involvement of *S. acidaminiphila* in the dissipation of IBP. Interestingly, several bacterial species belonging to the genus *Stenotrophomonas* have been reported as degrading microorganisms of other non-steroidal anti-inflammatory drugs such as naproxen and diclofenac [59,85]. However, the relative abundance of *S. acidaminiphila* decreased again 2.9 times at 7 days. This could be due to the presence of other degrading strains in the consortium C7 that remove IBP more efficiently from the culture medium, thus decreasing or avoiding the proliferation of *S. acidaminiphila* at 7 days.

3.4. Isolation and characterization of potential Ibuprofen degrader bacterial strains in consortium C7

After bacterial community composition analysis of consortium C7, the isolation of potential bacterial degraders of IBP to be used in

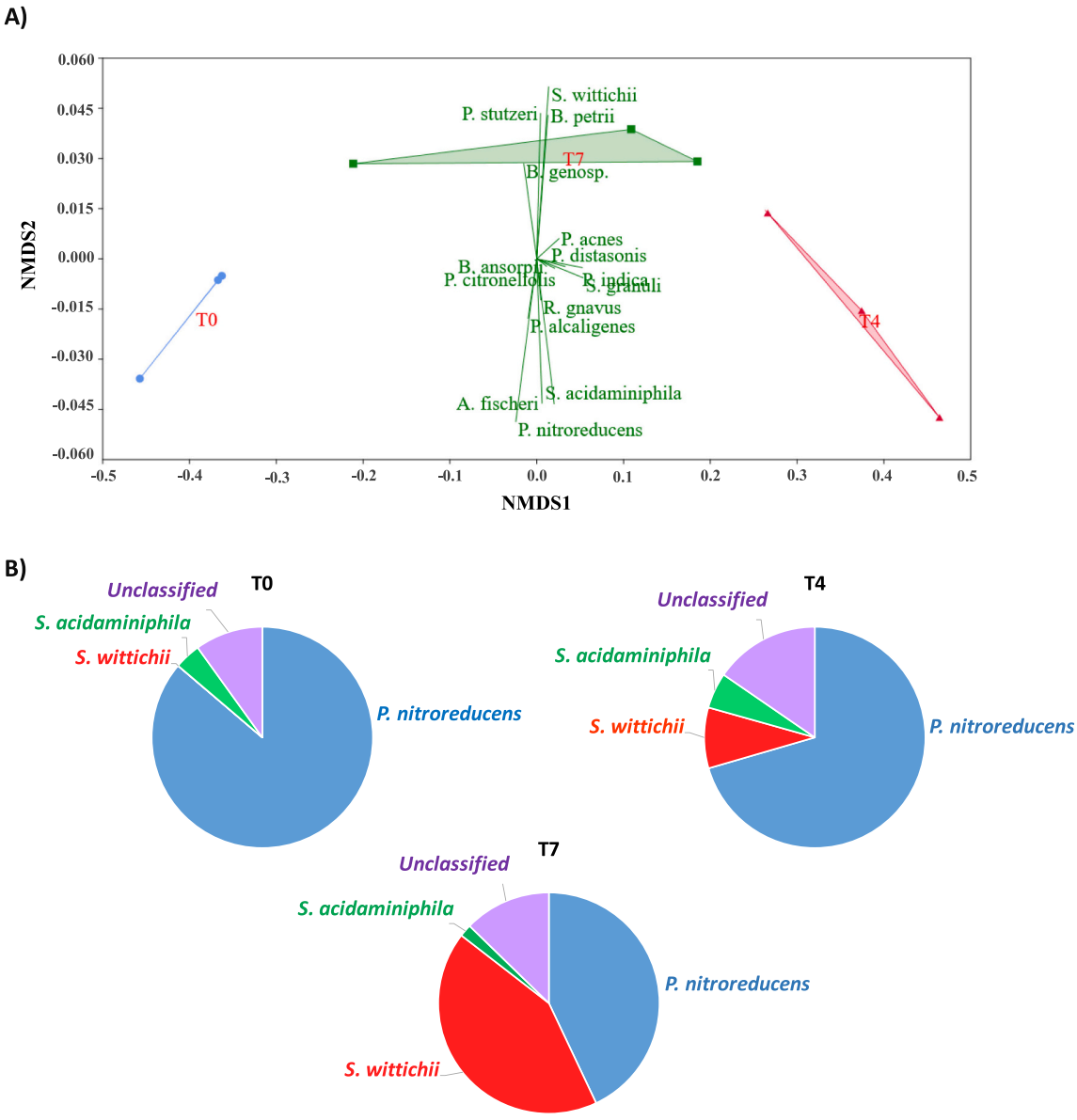

Fig. 8. Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis distances of the microbial community constrained by all species detected in the consortium C7 (A), and relative abundance of the most dominant bacterial species (>1% in any sample) detected in the consortium C7 during the acclimation process at 0, 4 and 7 days (B).

Table 1
Phylogenetic affiliations of bacterial strains isolated from ibuprofen enrichment cultures of sewage sludge.

Strain (accession number)	*NCBI affiliation (accession number)*	*Similarity (%)*	*Class; order; family; genus*
CSW06 (OQ859973)	*Brucella tritici (AM490635.1)*	*99.93*	*Alphaproteobacteria; Hyphomicrobiales; Brucellaceae; Brucella*
CSW07 (OQ859974)	*Bordetella petrii (FJ577503.1)*	*100*	*Betaproteobacteria; Burkholderiales; Alcaligenaceae; Bordetella*
CSW08 (OQ859975)	*Microbacterium paraoxydans (MK294281.1)*	*100*	*Actinomycetia; Micrococcales; Microbacteriaceae; Microbacterium*
CSW09 (OQ859976)	*Pseudomonas citronellolis (MN173542.1)*	*100*	*Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas*
CSW10 (OQ859977)	*Stenotrophomonas acidaminiphila (LT223687.1)*	*100*	*Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Stenotrophomonas*
CSW11 (OQ859978)	*Labrys neptuniae (NR_043801.1)*	*100*	*Alphaproteobacteria; Hyphomicrobiales; Xanthobacteraceae; Labrys*
CSW12 (OQ987996)	*Shinella zoogloeoides (KX161838.1)*	*100*	*Alphaproteobacteria; Hyphomicrobiales; Rhizobiaceae; Shinella*
CSW13 (OQ987997)	*Pseudomonas nitroreducens (CP049140.1)*	*99.85*	*Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas*
CSW14 (OQ987998)	*Pseudomonas citronellolis (MN173542.1)*	*99.62*	*Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas*
CSW15 (OQ987999)	*Achromobacter denitrificans (CP053987.1)*	*99.85*	*Betaproteobacteria; Burkholderiales; Alcaligenaceae; Achromobacter*
CSW16 (OQ988000)	*Pseudomonas citronellolis (MN173542.1)*	*99.78*	*Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas*
CSW17 (OQ988001)	*Pseudomonas citronellolis (MN173542.1)*	*99.85*	*Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas*
CSW18 (OQ988002)	*Curtobacterium flaccumfaciens (MF101085.1)*	*99.92*	*Actinomycetia; Micrococcales; Microbacteriaceae; Curtobacterium*

bioremediation processes was carried out through the enrichment culture using IBP as the only carbon and energy source. Thirteen bacterial strains were isolated from C7 consortium (Table 1), belonging three of them to the class Alfaproteobacteria (*Brucella tritici* CSW06, *Labrys neptuniae* CSW11, *Shinella zoogloeoides* CSW12), two of them to Betaproteobacteria (*Bordetella petrii* CSW07, *Achromobacter denitrificans* CSW15), 6 belonged to the class Gammaproteobacteria (*Stenotrophomonas acidaminiphila* CSW10, *Pseudomonas nitroreducens* CSW13, and 4 of them *Pseudomonas citronellolis* CSW09, CSW14, CSW16, CSW17), and two to Actinomycetia (*Microbacterium paraoxydans* CSW08 and *Curtobacterium flaccumfaciens* CSW18). Their accession numbers in the NCBI database and the percentage of similarity with the closest match of the 16 s rRNA gene sequences are also shown in Table 1.

IBP biodegradation in aqueous solution by bacteria isolated from C7 consortium after 14 days' incubation was carried out using 10 mg L^{-1} IBP as monosubstrate. Only *L. Neptuniae* was able to degrade IBP reaching an extent of degradation about 26%. IBP degradation by the rest of bacterial strains was similar to that obtained in the non-inoculated systems. To study the possibility of cometabolic degradation of these bacterial strains glucose was used as external carbon source. Environmental significance of cometabolism lies in the capacity of conversion of the target organic pollutant (which does not support growth) to products that accumulate and often are toxic products. However, when a second substrate used as C and energy sources is added, this will provoke an increase in the degradation rate of the target organic pollutant by cometabolism, due to a biomass increase and the generation of enzymes or cofactors required for its transformation [38, 52]. Fig. 9 shows the percentage of IBP degraded with the following decreasing order: 100%, *L. neptuniae*; 58%, *M. paraoxydans*; 45%, *A. denitrificans*; 33%, *P. citronellolis*; 26%, *B. tritici*, and the rest of strains degraded $\leq$ 20%. It indicated that an effective degradation of IBP occurs by cometabolism with glucose by some bacterial strains that had not been previously described as IBP-degraders. Interestingly, several of the isolated bacterial strains belong to the genera *Labrys, Achromobacter and Stenotrophomonas*, whose relative abundance was positively affected at different times during the acclimatization process (Fig. 7C). On the other hand, although *Bordetella petrii* showed greater influence at 7 days in the NMDS analysis (Fig. 8A), the bacterial strain *B. petrii* CSW07 only degraded 19.4% of IBP after 14 days (Fig. 9), which could suggest the need of other degrading bacteria to completely eliminate IBP. In fact, a bacterial consortium consisting of two *Bordetella petrii* strains and *Achromobacter xyloxidans* was related to the biodegradation of pesticides [17].

As far as we know, except *P. citronellolis* and *M. paraoxydans*, none of the other bacterial strains isolated in the present study have been previously described as IBP degraders. Very rencently, Wittich et al. [83] observed that *P. citronellolis* degraded IBP (but only acting as a consortium of three *P. citronellolis* (RW422, RW423, RW 424). Other *pseudomonas* has been recently proved to degrade IBP: *Pseudomonas* sp. M20 [14]. Show et al. [74] also observed that *M. paraoxydans* increased IBP degradation in the presence of yeast, although no other *Microbacterium* had been described for that purpose.

On the contrary, *L. neptuniae*, has not been described previously as IBP degrader, although the species *L. portucalensis* F11 has been demonstrated to degrade a wide variety of pharmaceutical products, such as ofloxacin, norfloxacin and ciprofloxacin [5], fluoxetine [49], diclofenac [50], or carbamazepine [12]. Something similar occurs with *S. zoogloeoides* and *P. nitroreducens*, which have not been described as IBP degraders, but strains isolated from WWTP sludge were shown to degrade the synthetic oestrogen 17α-ethinylestradiol [57] and fluoxetine [58]. *Achromobacter denitrificans* PR1 has also not been described as IBP degrader, but improved the removal of sulfonamides group pharmaceuticals [56,67,66]. On the contrary, as far as we know, in the cases of *B. tritici, B. petri, S. acidaminiphila* and *C. flaccumfaciens* there is no information as IBP degraders, and even the respective genus *Brucella, Bordetella, Stenotrophomonas or Curtobacterium* have not been described either.

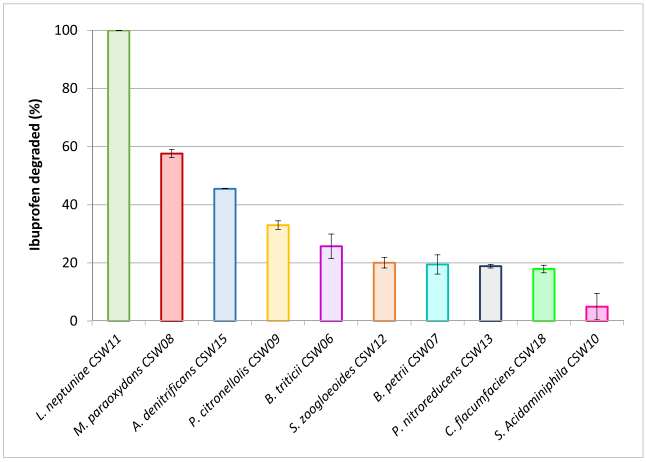

Fig. 9. Ibuprofen (10 mg L^{-1}) biodegradation by bacterial strains isolated from C7 consortium incubated for 14 days in the presence of glucose. Error bars show standard deviation.

4. Conclusions

The biodegradation in solution of a wide variety of IBP concentrations (1–1000 mg L^{-1}) by consortium C7 was carried out, reaching 100% degradation between 6 h and 30 h. The capacity of C7 for IBP mineralization (about 66% in 3 days) was also demonstrated. C7 shows greater potential for IBU degradation than most of the others consortia previously studied. Moreover, when IBP is adsorbed on sewage sludge, inoculation with C7 produced the biodegradation of 90% IBP, in comparison to 30% dissipated in the non-bioaugmented sludge. All these data indicated the high capacity of consortium C7 for not only biodegrade but also mineralize IBP, even strongly adsorbed on sewage sludge.

Bacterial community of consortium C7 suffered a shift after IBP degradation. The genera *Sphingomonas* increased drastically, being potentially involved in IBP degradation and mineralization. The relative abundance of the genera *Pseudoxanthomonas*, *Labrys*, *Pandoraea* and *Pigmentaphaga* also increased. Fifteen bacterial species were detected in the consortium C7, but the relative abundance of *Sphingomonas wittichii* increased drastically (from 0 to >40%) when IBP was completely degraded, indicating that it is the main bacterial specie responsible for IBP degradation.

Bacterial strains with the ability to grow in the presence of IBP were isolated from C7: *B. tritici, L. neptuniae, S. zoogloeoides, B. petrii, A. denitrificans, S. acidaminiphila, P. nitroreducens, P. citronellolis, M. paraoxydans,* and *C. flaccumfaciens*. Only *L. neptuniae* was able to degrade IBP as monosubstrate, but when glucose was present all of them showed IBP degradation, being the most effective *L. neptuniae*. Except *P. citronellolis* and *M. paraoxydans*, it is the first time that the other 8 bacterial strains are described as IBP degraders.

Environmental Implication

The wide use of ibuprofen (IBP) involves ecological risks, being considered an emerging pollutant. IBP excreted by humans ends up in WWTPs, but partially remains not only in the effluent waters used for irrigation but also in sewage sludge used as fertilizer. WWTPs is the unique opportunity to remove IBP from both matrices before its dispersion in the environment. Consortium C7 isolated from sewage sludge reached an IBP degradation higher than other IBP-degrading consortia in aqueous solution and, for the first time, in sewage sludge. Bacterial community changes were studied and new effective IBP-degrading bacteria were also isolated.

CRediT authorship contribution statement

I. Aguilar-Romero: Design of experiment, Investigation, Methodology, Data curation, Writing, Review. **F. Madrid:** Design of experiment, Investigation, Methodology, Data curation, Review. **J. Villaverde:** Design of experiment, Investigation, Supervision, Data curation, Review. **E. Morillo:** Conceptualization, Design of experiment, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition.

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.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.132970.

References

[1] Abegglen, C., Joss, A., McArdell, C.S., Fink, G., Schlusener, M.P., Ternes, T.A., et al., 2009. The fate of selected micropollutants in a single-house MBR. Environ Int 43, 2036–2046. https://doi.org/10.1016/j.watres.2009.02.005.

[2] Aguilar-Romero, I., Romero, E., Wittich, R.M., van Dillewijn, P., 2020. Bacterial ecotoxicity and shifts in bacterial communities associated with the removal of ibuprofen, diclofenac and triclosan in biopurification systems. Sci Total Environ, 140461. https://doi.org/10.1016/j.scitotenv.2020.140461.

[3] Aguilar-Romero, I., De la Torre-Zúñiga, J., Quesada, J.M., Haïdour, A., O'Connell, G., McAmmond, B.M.Van Hamme, et al., 2021. Effluent decontamination by the ibuprofen-mineralizing strain, Sphingopyxis granuli RW412: metabolic processes. Environ Pollut, 116536. https://doi.org/10.1016/j.envpol.2021.116536.

[4] Ajibola, A.S., Adebiyi, A.O., Nwaeke, D.O., Ajibola, F.O., Adewuyi, G.O., 2021. Analysis, occurrence and ecological risk assessment of diclofenac and ibuprofen residues in wastewater from three wastewater treatment plants in south-western Nigeria. J Appl Sci Environ Manag 25, 333–340. https://doi.org/10.4314/jasem.v25i3.5.

[5] Amorim, C.L., Moreira, I.S., Maia, A.S., Tiritan, M.E., Castro, P.M.L., 2014. Biodegradation of ofloxacin, norfloxacin, and ciprofloxacin as single and mixed substrates by *Labrys portucalensis* F11. Appl Microbiol Biotechnol 98, 3181–3190. https://doi.org/10.1007/s00253-013-5333-8.

[6] Aulestia, M., Flores, A., Acosta-Jurado, S., Santero, E., Camacho, E.M., 2022. Genetic characterization of the ibuprofen-degradative pathway of rhizorhabdus wittichii MPO218. Appl Environ Microbiol 88, e00388-22. https://doi.org/10.1128/aem.00388-22.

[7] Aulestia, M., Flores, A., Mangas, E.L., Pérez-Pulido, A.J., Santero, E., Camacho, E.M., 2021. Isolation and genomic characterization of the ibuprofen-degrading bacterium *Sphingomonas* strain MPO218. Environ Microbiol 23, 267–280. https://doi.org/10.1111/1462-2920.15309.

[8] Austin, T., Bregoli, F., Höhne, D., Hendriks, A.J., Ragas, A.M.J., 2022. Ibuprofen exposure in Europe; ePiE as an alternative to costly environmental monitoring. Environ Res 209, 112777. https://doi.org/10.1016/j.envres.2022.112777.

[9] Aydin, S., 2016. Enhanced biodegradation of antibiotic combinations via the sequential treatment of the sludge resulting from pharmaceutical wastewater treatment using white-rot fungi *Trametes versicolor* and Bjerkandera adusta. Appl Microbiol Biotechnol 100, 6491–6499. https://doi.org/10.1007/s00253-016-7473-0.

[10] Balciunas, E.M., Kappelmeyer, U., Harms, H., Heipieper, H.J., 2020. Increasing ibuprofen degradation in constructed wetlands by bioaugmentation with gravel containing biofilms of an ibuprofen-degrading *Sphingobium yanoikuyae*. Eng Life Sci 20, 160–167. https://doi.org/10.1002/elsc.201900097.

[11] Bankole, P.O., Adekunle, A.A., Jeon, B.H., Govindwar, S.P., 2020. Novel cobiomass degradation of NSAIDs by two wood rot fungi, Ganoderma applanatum and Laetiporus sulphureus: ligninolytic enzymes induction, isotherm and kinetic studies. Ecotoxicol Environ Saf 203, 110997. https://doi.org/10.1016/j.ecoenv.2020.110997.

[12] Bessa, V.S., Moreira, I.S., Murgolo, S., Mascolo, G., Castro, P.M.L., 2019. Carbamazepine is degraded by the bacterial strain *Labrys portucalensis* F11. Sci Total Environ 690, 739–747. https://doi.org/10.1016/j.scitotenv.2019.06.461.

[13] Boix, C., Ibáñez, M., Sancho, J.V., Parsons, J.R., Voogt, P., Hernández, F., 2016. Biotransformation of pharmaceuticals in surface water and during waste water treatment: identification and occurrence of transformation products. J Hazard Mater 302, 175–187. https://doi.org/10.1016/j.jhazmat.2015.09.053.

[14] Chen, R., Huang, J., Li, X., Yang, C., Wu, X., 2023. Functional characterization of an efficient ibuprofen-mineralizing bacterial consortium. J Hazard Mater 447, 130751. https://doi.org/10.1016/j.jhazmat.2023.130751.

[15] Chopra, S., Kumar, D., 2020. Ibuprofen as an emerging organic contaminant in environment, distribution and remediation. Heliyon 6, e04087. https://doi.org/10.1016/j.heliyon.2020.e04087.

[16] Chopra, S., Kumar, D., 2022. Characterization and biodegradation of ibuprofen by Bacillus siamensis strain DSI-1 isolated from wastewater. Rend Lince– 33, 643–652. https://doi.org/10.1007/s12210-022-01085-6.

[17] Cycoń, M., Mrozik, A., Piotrowska-Seget, Z., 2017. Bioaugmentation as a strategy for the remediation of pesticide-polluted soil: a review. Chemosphere 172, 52–71. https://doi.org/10.1016/j.chemosphere.2016.12.129.

[18] Davids, M., Gudra, D., Radovica-Spalvina, I., Fridmanis, D., Bartkevics, V., Muter, O., 2017. The effects of ibuprofen on activated sludge: Shift in bacterial community structure and resistance to ciprofloxacin. J Hazard Mater 340, 291–299. https://doi.org/10.1016/j.jhazmat.2017.06.065.

[19] DG Env, 2020. Priority Substances Review-Next steps. https://circabc.europa.eu/sd/a/97910629–048e-4b2b-90a2-fe520aab2adc/WGChem2020Jan(7)PrioritySubstancesReview-nextsteps.pdf.

[20] Di Baccio, D., Pietrini, F., Bertolotto, P., Pérez, S., Barcelò, D., Zacchini, M., et al., 2017. Response of *Lemna gibba* L. to high and environmentally relevant concentrations of ibuprofen: removal, metabolism and morpho-physiological traits for biomonitoring of emerging contaminants. Sci Total Environ 584–585, 363–373. https://doi.org/10.1016/j.scitotenv.2016.12.191.

[21] Du, M.S., Chen, K.P., Lin, Y.P., 2019. Degradation of ibuprofen and acetylsulfamethoxazole by multi-walled carbon nanotube catalytic ozonation: surface properties, kinetics and modeling. Environ Sci Water Res Technol 5, 1758–1768. https://doi.org/10.1039/C9EW00492K.

[22] EC, 2013. European Commission, European Parliament Legislative Resolution of 2 July 2013 on the Proposal for a Directive of the European Parliament and of the Council Amending Directives 2000/60/EC and 2008/105/EC as Regards Priority Substances in the Field of Water Policy.

[23] Ellepola, N., Ogas, T., Turner, D.N., Gurung, R., Maldonado-Torres, S., Tello-Aburto, R., et al., 2020. A toxicological study on photo-degradation products of environmental ibuprofen: ecological and human health implications. Ecotoxicol Environ Saf 188, 109892. https://doi.org/10.1016/j.ecoenv.2019.109892.

[24] Estevez, E., Hernandez-Moreno, J.M., Fernandez-Vera, J.R., Palacios-Diaz, M.P., 2014. Ibuprofen adsorption in four agricultural volcanic soils. Sci Total Environ 468–469, 406–414. https://doi.org/10.1016/j.scitotenv.2013.07.068.

[25] Ferrando-Climent, L., Collado, N., Buttiglieri, G., Gros, M., Rodriguez-Roda, I., Rodriguez-Mozaz, S., et al., 2012. Comprehensive study of ibuprofen and its metabolites in activated sludge batch experiments and aquatic environment. Sci Total Environ 438, 404–413. https://doi.org/10.1016/j.scitotenv.2012.08.073.

[26] Ferrer-Polonio, E., Fernández-Navarro, J., Iborra-Clar, M.I., Alcaina-Miranda, M.I., Mendoza-Roca, J.A., 2020. Removal of pharmaceutical compounds commonly-found in wastewater through a hybrid biological and adsorption process. J Environ Manag, 110368. https://doi.org/10.1016/j.jenvman.2020.110368.

[27] Fortunato, M.S., Fuentes Abril, N.P., Martinefski, M., Trípodi, V., Papalia, M., Rádice, M., et al., 2016. Aerobic degradation of ibuprofen in batch and continuous reactors by an indigenous bacterial community. Environ Technol 37, 2617–2626. https://doi.org/10.1080/09593330.2016.1156773.

[28] Gongora, J.F., Elizondo, P., Hernandez-Ramírez, A., 2017. Photocatalytic degradation of ibuprofen using TiO2 sensitized by Ru(II) polyaza complexes. Photochem Photobiol Sci 16, 31–37. https://doi.org/10.1039/c6pp00222f.

[29] Hesham, A.E.-L., Khan, S., Tao, Y., Li, D., Zhang, Y., Yang, M., 2012. Biodegradation of high molecular weight PAHs using isolated yeast mixtures: application of meta-genomic methods for community structure analyses. Environ Sci Pollut Res 19, 3568–3578. https://doi.org/10.1007/s11356-012-0919-8.

[30] Hörsing, M., Ledin, A., Grabic, R., Fick, J., Tysklind, M., Jansen, J.C., et al., 2011. Determination of sorption of seventy-five pharmaceuticals in sewage sludge. Water Res 45, 4470–4482. https://doi.org/10.1016/j.watres.2011.05.033.

[31] Huang, C.Y., Fu, L.H., Sung, M.H., Huang, C.F., Wu, J.P., Kuo, H.W., 2020. Ibuprofen biodegradation by hospital, municipal, and distillery activated sludge. Environ Technol 41, 171–180. https://doi.org/10.1080/09593330.2018.1493146.

[32] ISO 11348-3, 2007. Water quality - determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) Part 3: method using freeze-dried bacteria. Int Organ Stand. ⟨https://www.iso.org/standard/40518.html⟩.

[33] Jia, Y., Yin, L., Khanal, S.K., Zhang, H., Oberoi, A.S., Lu, H., 2020. Biotransformation of ibuprofen in biological sludge systems: investigation of performance and mechanisms. Water Res, 115303. https://doi.org/10.1016/j.watres.2019.115303.

[34] Jiang, C., Geng, J., Hu, H., Ma, H., Gao, X., Ren, H., 2017. Impact of selected non-steroidal anti-inflammatory pharmaceuticals on microbial community assembly and activity in sequencing batch reactors. PloS One 12, e0179236. https://doi.org/10.1371/journal.pone.0179236.

[35] Jurado, A., Vázquez-Sune, E., Pujades, E., 2021. Urban groundwater contamination by nonsteroidal anti-inflammatory drugs. Water 13, 720. https://doi.org/10.3390/w13050720.
[36] Kanteraki, A.E., Isari, E.A., Svarnas, P., Kalavrouziotis, I.K., 2022. Biosolids: the Trojan horse or the beautiful Helen for soil fertilization? Sci Total Environ 83915, 156270. https://doi.org/10.1016/j.scitotenv.2022.156270.
[37] Lachassagne, D., Soubrand, M., Casellas, M., Gonzalez-Ospina, A., Dagot, C., 2015. Impact of sludge stabilization processes and sludge origin (urban or hospital) on the mobility of pharmaceutical compounds following sludge landspreading in laboratory soil-column experiments. Environ Sci Pollut Res 22, 17135–17150. https://doi.org/10.1007/s11356-015-4918-4.
[38] Lara-Moreno, A., Aguilar-Romero, I., Rubio-Bellido, M., Madrid, F., Villaverde, J., Santos, J.L., et al., 2022. Novel nonylphenol-degrading bacterial strains isolated from sewage sludge: application in bioremediation of sludge. Sci Total Environ 847, 157647. https://doi.org/10.1016/j.scitotenv.2022.157647.
[39] Larsen, S.B., Karakashev, D., Angelidaki, I., Schmidt, J.E., 2009. Ex-situ bioremediation of polycyclic aromatic hydrocarbons in sewage sludge. J Hazard Mater 164, 1568–1572. https://doi.org/10.1016/j.jhazmat.2008.08.067.
[40] Lin, K., Gan, J., 2011. Sorption and degradation of wastewater-associated non-steroidal anti-inflammatory drugs and antibiotics in soils. Chemosphere 83, 240–246. https://doi.org/10.1016/j.chemosphere.2010.12.083.
[41] Liu, Y., Chang, H., Li, Z., Feng, Y., Cheng, D., Xue, J., 2017. Biodegradation of gentamicin by bacterial consortia AMQD4 in synthetic medium and raw gentamicin sewage. Sci Rep 7, 11004. https://doi.org/10.1038/s41598-017-11529-x.
[42] Lu, Z., Sun, W., Li, C., Ao, X., Yang, C., Li, S., 2019. Bioremoval of non-steroidal anti-inflammatory drugs by Pseudoxanthomonas sp. DIN-3 isolated from biological activated carbon process. Water Res 161, 459–472. https://doi.org/10.1016/j.watres.2019.05.065.
[43] Madrid, F., Rubio-Bellido, M., Villaverde, J., Peña, A., Morillo, E., 2019. Natural and assisted dissipation of polycyclic aromatic hydrocarbons in a long-term co-contaminated soil with creosote and potentially toxic elements. Sci Total Environ 660, 705–714. https://doi.org/10.1016/j.scitotenv.2018.12.376.
[44] Malvar, J.L., Santos, J.L., Martín, J., Aparicio, I., Alonso, E., 2020. Occurrence of the main metabolites of pharmaceuticals and personal care products in sludge stabilization treatments. Waste Manag 116, 22–30. https://doi.org/10.1016/j.wasman.2020.07.051.
[45] Marchlewicz, A., Guzik, U., Wojcieszynska, D., 2015. Over-the-counter monocyclic nonsteroidal anti-inflammatory drugs in environment–sources, risks, biodegradation. Water Air Soil Pollut 226, 355. https://doi.org/10.1007/s11270-015-2622-0.
[46] Marchlewicz, A., Guzik, U., Smulek, W., Wojcieszynska, D., 2017. Exploring the degradation of ibuprofen by Bacillus thuringiensis B1 (2015b): the new pathway and factors affecting degradation. Molecules 22, 1676. https://doi.org/10.3390/molecules22101676.
[47] Marchlewicz, A., Guzik, U., Hupert-Kocurek, K., Wojcieszyńska, D., 2023. Evaluation of the defined bacterial consortium efficacy in the biodegradation of NSAIDS. Molecules 28, 2185. https://doi.org/10.3390/molecules28052185.
[48] Mejías, C., Martín, J., Santos, J.L., Aparicio, I., Alonso, E., 2021. Occurrence of pharmaceuticals and their metabolites in sewage sludge and soil: A review on their distribution and environmental risk assessment. Trends Environ Anal Chem 30, e00125. https://doi.org/10.1016/j.teac.2021.e00125.
[49] Moreira, I.S., Ribeiro, A.R., Afonso, C.M., Tiritan, M.E., Castro, P.M., 2014. Enantioselective biodegradation of fluoxetine by the bacterial strain *Labrys portucalensis* F11. Chemosphere 111, 103–111. https://doi.org/10.1016/j.chemosphere.2014.03.022.
[50] Moreira, I.S., Bessa, V.S., Murgolo, S., Piccirillo, C., Mascolo, G., Castro, P.M., 2018. Biodegradation of diclofenac by the bacterial strain *Labrys portucalensis* F11. Ecotoxicol Environ Saf 152, 104–113. https://doi.org/10.1016/j.ecoenv.2018.01.040.
[51] Morillo, E., Villaverde, J., 2017. Advanced technologies for the remediation of pesticide contaminated soils. Sci Total Environ 586, 576–597. https://doi.org/10.1016/j.scitotenv.2017.02.020.
[52] Mulkiewicz, E., Wolecki, D., Świacka, K., Kumirska, J., Stepnowski, P., Caban, M., 2021. Metabolism of non-steroidal anti-inflammatory drugs by non-target wild-living organisms. Sci Total Environ, 148251. https://doi.org/10.1016/j.scitotenv.2021.148251.
[53] Murdoch, R.W., Hay, A.G., 2005. Formation of catechols via removal of acid side chains from ibuprofen and related aromatic acids. Appl Environ Microbiol 71, 6121–6125. https://doi.org/10.1128/AEM.71.10.6121-6125.2005.
[54] Murdoch, R.W., Hay, A.G., 2013. Genetic and chemical characterization of ibuprofen degradation by Sphingomonas Ibu-2. Microbiology 159, 621–632. https://doi.org/10.1099/mic.0.062273-0.
[55] Navrozidou, E., Remmas, N., Melidis, P., Karpouzas, D.G., Tsiamis, G., Ntougias, S., 2019. Biodegradation potential and diversity of diclofenac-degrading microbiota in an immobilized cell biofilter. Processes 7, 554. https://doi.org/10.3390/pr7090554.
[56] Nguyen, P.Y., Silva, A.F., Reis, A.C., Nunes, O.C., Rodrigues, A.M., Rodrigues, J.E., et al., 2019. Bioaugmentation of membrane bioreactor with *Achromobacter denitrificans* strain PR1 for enhanced sulfamethoxazole removal in wastewater. Sci Total Environ 648, 44–55. https://doi.org/10.1016/j.scitotenv.2018.08.100.
[57] Palma, T.L., Shylova, A., Costa, M.C., 2021. Isolation and characterization of bacteria from activated sludge capable of degrading 17α-ethinylestradiol, a contaminant of high environmental concern. Microbiology 167, 001038. https://doi.org/10.1099/mic.0.001038.
[58] Palma, T.L., Shylova, A., Dias Carlier, J., Costa, M.C., 2021. An autochthonous aerobic bacterial community and its cultivable isolates capable of degrading fluoxetine. J Chem Technol Biotechnol 96, 2813–2826. https://doi.org/10.1002/jctb.6829.
[59] Pápai, M., Benedek, T., Táncsics, A., Bornemann, T.L., Plewka, J., Probst, A.J., et al., 2023. Selective enrichment, identification, and isolation of diclofenac, ibuprofen, and carbamazepine degrading bacteria from a groundwater biofilm. Environ Sci Pollut Res 30, 44518–44535. https://doi.org/10.1007/s11356-022-24975-6.
[60] Parolini, M., 2020. Toxicity of the nonsteroidal anti-inflammatory drugs (NSAIDs) acetylsalicylic acid, paracetamol, diclofenac, ibuprofen and naproxen towards freshwater invertebrates: a review. Sci Total Environ 740, 140043. https://doi.org/10.1016/j.scitotenv.2020.140043.
[61] Praveenkumarreddy, Y., Vimalkumar, K., Ramaswamy, B.R., Kumar, V., Singhal, R. K., Basu, H., et al., 2021. Assessment of non-steroidal anti-inflammatory drugs from selected wastewater treatment plants of Southwestern India. Emerg Contam 7, 43–51. https://doi.org/10.1016/j.emcon.2021.01.001.
[62] Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41, D590–D596. https://doi.org/10.1093/nar/gks1219.
[63] Quintana, J.B., Weiss, S., Reemtsma, T., 2005. Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor. Water Res 39, 2654–2664. https://doi.org/10.1016/j.watres.2005.04.068.
[64] Quintelas, C., Mesquita, D.P., Torres, A.M., Costa, I., Ferreira, E.C., 2020. Degradation of widespread pharmaceuticals by activated sludge: kinetic study, toxicity assessment, and comparison with adsorption processes. J Water Process Eng 33, 101061. https://doi.org/10.1016/j.jwpe.2019.101061.
[65] Rastogi, A., Tiwari, M.K., Ghangrekar, M.M., 2021. A review on environmental occurrence, toxicity and microbial degradation of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). J Environ Manag 30015, 113694. https://doi.org/10.1016/j.jenvman.2021.113694.
[66] Reis, A.C., Cvancarova, M., Liu, Y., Lenz, M., Hettich, T., Kolvenbach, B.A., et al., 2018. Biodegradation of sulfamethoxazole by a bacterial consortium of *Achromobacter denitrificans* PR1 and *Leucobacter* sp. GP. Appl Microbiol Biotechnol 102, 10299–10314. https://doi.org/10.1007/s00253-018-9411-9.
[67] Reis, P.J.M., Reis, A.C., Ricken, B., Kolvenbach, B.A., Manaia, C.M., Corvini, P.F.X., et al., 2014. Biodegradation of sulfamethoxazole and other sulfonamides by *Achromobacter denitrificans* PR1. J Hazard Mat 280, 741–749. https://doi.org/10.1016/j.jhazmat.2014.08.039.
[68] Rodriguez-Rodriguez, C.E., Jelić, A., Llorca, M., Farré, M., Caminal, G., Petrović, M., et al., 2011. Solid-phase treatment with the fungus *Trametes versicolor* substantially reduces pharmaceutical concentrations and toxicity from sewage sludge. Bioresour Technol 102, 5602–5608. https://doi.org/10.1016/j.biortech.2011.02.029.
[69] Rodríguez-Rodríguez, C.E., Barón, E., Gago-Ferrero, P., Jelić, A., Llorca, M., Farré, M., et al., 2012. Removal of pharmaceuticals, polybrominated flame retardants and UV-filters from sludge by the fungus *Trametes versicolor* in bioslurry reactor. J Hazard Mater 233–234, 235–243. https://doi.org/10.1016/j.jhazmat.2012.07.024.
[70] Rodríguez-Rodríguez, C.E., Lucas, D., Barón, E., Gago-Ferrero, P., Molins-Delgado, D., Rodríguez-Mozaz, S., et al., 2014. Re-inoculation strategies enhance the degradation of emerging pollutants in fungal bioaugmentation of sewage sludge. Bioresour Technol 168, 180–189. https://doi.org/10.1016/j.biortech.2014.01.124.
[71] Rutere, C., Knoop, K., Posselt, M., Ho, A., Horn, M.A., 2020. Ibuprofen degradation and associated bacterial communities in hyporheic zone sediments. Microorganisms 8, 1245. https://doi.org/10.3390/microorganisms8081245.
[72] Sharma, K., Kaushik, G., Thotakura, N., Raza, K., Sharma, N., Nimesh, S., 2019. Fate of ibuprofen under optimized batch biodegradation experiments using *Micrococcus yunnanensis* isolated from pharmaceutical sludge. Int J Environ Sci Technol 16, 8315–8328. https://doi.org/10.1007/s13762-019-02400-9.
[73] Show, S., Chakraborty, P., Karmakar, B., Halder, G., 2021. Sorptive and microbial riddance of micro-pollutant ibuprofen from contaminated water: a state of the art review. Sci Total Environ 786, 147327. https://doi.org/10.1016/j.scitotenv.2021.147327.
[74] Show, S., Sarkar, P., Barman, S., Halder, G., 2023. Microbial remediation of ibuprofen contaminated water using novel isolate *Microbacterium paraoxydans*. Chem Pap 77, 517–531. https://doi.org/10.1007/s11696-022-02499-0.
[75] Taha, M., Shahsavari, E., Aburto-Medina, A., Foda, M., Clarke, B., Roddick, F., et al., 2018. Bioremediation of biosolids with Phanerochaete chrysosporium culture filtrates enhances the degradation of polycyclic aromatic hydrocarbons (PAHs). Appl Soil Ecol 124, 163–170. https://doi.org/10.1016/j.apsoil.2017.11.002.
[76] Thalla, A.K., Vannarath, A.S., 2020. Occurence and environemntal risks of nonsteroidal anti-inflammatory drugs in urban wastewater in the southwest monsson region of India. Environ Monit Assess 192, 193. https://doi.org/10.1007/s10661-020-8161-1.
[77] Urase, T., Kikuta, T., 2005. Separate estimation of adsorption and degradation of pharmaceutical substances and estrogens in the activated sludge process. Water Res 39, 1289–1300. https://doi.org/10.1016/j.watres.2005.01.015.
[78] Vargas-Ordóñez, A., Aguilar-Romero, I., Villaverde, J., Madrid, F., Morillo, E., 2023. Isolation of novel bacterial strains *Pseudomonas extremaustralis* CSW01 and *Stutzerimonas stutzeri* CSW02 from sewage sludge for paracetamol biodegradation. Microorganisms 11, 196. https://doi.org/10.3390/microorganisms11010196.

[79] Vilaplana, M., Rodríguez-Rodríguez, C.E., Barón, E., Gorga, M., Sarrà, M., Caminal, G., et al., 2015. Biodegradation of polybrominated diphenyl ethers in liquid media and sewage sludge by *Trametes versicolor*. Int J Environ Res 9, 273–280.
[80] Wang, Z., Liang, B., Hou, Y., Li, S., Xie, L., Peng, L., et al., 2022. Weak electrostimulation enhanced the microbial transformation of ibuprofen and naproxen. Sci Total Environ 835, 155522. https://doi.org/10.1016/j.scitotenv.2022.155522.
[81] WFD, EC, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. J 327 (L).
[82] Wijaya, L., Alyemeni, M., Ahmad, P., Alfarhan, A., Barcelo, D., El-Sheikh, M.A., et al., 2020. Ecotoxicological effects of ibuprofen on plant growth of *Vigna unguiculata* L. Plants 9, 1473. https://doi.org/10.3390/plants9111473.
[83] Wittich, R.M., Haïdour, A., Aguilar-Romero, I., de la Torre-Zúñiga, J., van Dillewijn, P., 2023. Biodegradation of microtoxic phenylpropanoids (phenylpropanoic acid and ibuprofen) by bacteria and the relevance for their removal from wastewater treatment plants. Genes 14, 442. https://doi.org/10.3390/genes14020442.
[84] Wojcieszynska, D., Guzik, H., Guzik, U., 2022. Non-steroidal anti-inflammatory drugs in the era of the COVID-19 pandemic in the context of the human and the environment. Sci Total Environ 834, 155317. https://doi.org/10.1016/j.scitotenv.2022.155317.
[85] Wojcieszyńska, D., Domaradzka, D., Hupert-Kocurek, K., Guzik, U., 2014. Bacterial degradation of naproxen–undisclosed pollutant in the environment. J Environ Manag 145, 157–161. https://doi.org/10.1016/j.jenvman.2014.06.023.
[86] Wu, J., Liu, Z., Ma, Q., Dai, L., Dang, Z., 2023. Occurrence, removal and risk evaluation of ibuprofen and acetaminophen in municipal wastewater treatment plants: a critical review. Sci Total Environ, 164600. https://doi.org/10.1016/j.scitotenv.2023.164600.
[87] Xu, B., Xue, G., Yang, X., 2018. Isolation and application of an ibuprofen-degrading bacterium to a biological aerated filter for the treatment of micro-polluted water. Front Environ Sci Eng 12, 15. https://doi.org/10.1007/s11783-018-1080-5.
[88] Yu, T.H., Lin, A.Y.C., Panchangam, S.C., Hong, P.K.A., Yang, P.Y., Lin, C.F., 2011. Biodegradation and bio-sorption of antibiotics and non-steroidal anti-inflammatory drugs using immobilized cell process. Chemosphere 84, 1216–1222. https://doi.org/10.1016/j.chemosphere.2011.05.045.
[89] Zur, J., Pinski, A., Marchlewicz, A., Hupert-Kocurek, K., Wojcieszynska, D., Guzik, U., 2018. Organic micropollutants paracetamol and ibuprofen—toxicity, biodegradation, and genetic background of their utilization by bacteria. Environ Sci Pollut Res 25, 21498–21524. https://doi.org/10.1007/s11356-018-2517-x.
[90] Zwiener, C., Seeger, S., Glauner, T., Frimmel, F.H., 2002. Metabolites from the biodegradation of pharmaceutical residues of ibuprofen in biofilm reactors and batch experiments. Anal Bioanal Chem 372, 569–575. https://doi.org/10.1007/s00216-001-1210-x.