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Empirical Evidence and Mathematical Modelling of Carbamazepine Degradative Kinetics by a Wood-Rotting Microbial Consortium

N. González-Benítez[1] · M. C. Molina[1] · M. Arrayás[2]

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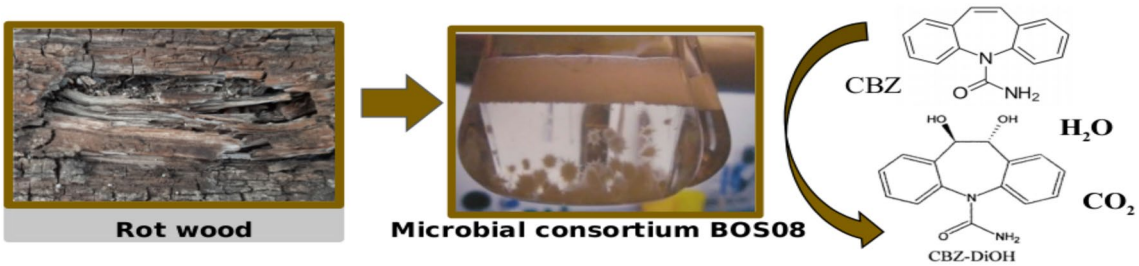
Abstract

An experimental evolution system with a wood-rotting microbial consortium (BOS08) has demonstrated the acquisition of a new ability to exploit a previously untapped carbon source, such as the recalcitrant carbamazepine (CBZ). The improved extraction method has provided an accurate CBZ depletion rate from BOS08 of $2.14 \pm 0.42 \times 10^{-3}$ h^{-1}. The consortium did not use cometabolism to process CBZ and the intermediate metabolite produced 10,11-dihydroxycarbamazepine was not pharmacologically active and toxic. The bacteria identification by massive sequencing (Illumina) confirmed the dominance of Proteobacteria Phylum such as genera *Cupriavidus* sp., *Sphingomonas* sp., *Delftia* sp., *Acinetobacter* sp. and *Methylobacterium* sp. coexisting through all biodegradation process. Based on biological principles, we model the consortium-CBZ kinetics with a set of nonlinear ordinary differential equations with logistic growth type terms. The use of experimental data combined with logistic growth models allow us to test new functional features acquired by the consortium.

Graphic Abstract



Keywords Wood-rotting consortium · Carbamazepine · *Proteobacteria* · Kinetics modelling

Statement of Novelty

Serial transfer experiment has imposed a selective pressure on the microbial consortium BOS08 to allow the enrichment and isolation of bacterial strains with the ability to degrade CBZ. The use of these experimental data combined with a mathematical model based on first principles permits the prediction of the biodegradation of recalcitrant pollutants such a CBZ.

Introduction

Emerging contaminants (ECs) are new synthetic or naturally occurring chemicals and include any microorganisms that are not regulated and whose impact on the environment and human health are poorly understood (UNESCO). These chemicals include pharmaceuticals and personal care product (PPCPs). They are biologically active, relatively persistent, and resistant to inactivation or degradation. The

✉ N. González-Benítez
natalia.gonzalez@urjc.es

[1] Departamento de Biología y Geología, Física y Química Inorgánica, Universidad Rey Juan Carlos, Tulipán s/n, 28933 Móstoles, Madrid, Spain

[2] Área de Electromagnetismo, Universidad Rey Juan Carlos, Tulipán s/n, 28933 Móstoles, Madrid, Spain

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increase of the world human population [1] makes these PPCPs produced and consumed annually in large quantities [2]. These pollutants reach natural waters through different mechanism such as urban wastewater from hospitals [3], excretion by the human body and direct disposal through the drainage system [4]. In semi-arid areas such as the Mediterranean [5], the use of water from waste water treatment plants (WWTPs) [6] for intensive agriculture [6] is another source of enviromental impact.

The high sensitivity of recent analytical techniques such as high-performance liquid chromatography–mass spectrometry (HPLC–MS), gas chromatography–mass spectrometry (GC–MS), Raman spectroscopy and nuclear magnetic resonance has allowed to detect the PPCPs at concentrations from 0.15 ng l^{-1} to 400 µg l^{-1} [7]. The low concentration of the PPCPs found in surface water, groundwater and water human consumption might pose potential risks to human health [8, 9]. WWTPs are not currently designed for complete remove capacity of recalcitrant PPCPs, showing low remediation efficiencies [10–12]. Recalcitrant PPCPs have hydrophobic characteristic, which favor the bioconcentration in the aquatic organisms [13] and the biomagnification at the upper trophic positions. Their continuous discharge, bioaccumulation and/or synergistic combination cause serious adverse effects to the environment and/or to humans [4, 14].

Many research studies concerning removal of PPCPs conduct physico-chemical processes [15] such as ozonation, Fe^{2+}/S_2O_8, UV/H_2O_2, photo-fenton procedure, electrochemical oxidation, photocatalysis [16, 17], activated carbon adsorption, advanced oxidation processes, nanofiltration, reverse osmosis and photodegradation [14]. Other biological alternatives can also achieve higher ECs removal as conventional activated sludge treatment, membrane bioreactor (MBR), constructed wetland, algae photobioreactor and stabilization ponds [15, 18]. From the energetic, economic and environmental point of view, the biological approaches are better than physico-chemical ones. The hydrophobic characteristic of some PPCPs favor the bioconcentration in the aquatic organisms [13] and the biomagnification at the upper trophic positions. Their continuous discharge, bioaccumulation and/or synergistic combination cause serious adverse effects to the environment and/or to humans [4, 14].

Currently, most of the WWTP are not designed to eliminate efficiently these ubiquitous drugs such as carbamazepine (CBZ) [11, 12] because they appear highly diluted and are recalcitrant [19, 20]. Despite this interest, there is still a need for efficient recalcitrant PPCPs biodegradation processes.

The CBZ is an anti-epileptic drug, considered priority pharmaceutical products (classified as Class I [21]), worldwide consumed (> 1000 tons per year) and highly persistent [12, 22]. A recent review about the pharmaceuticals of emerging concern in aquatic systems [10] has reported that still CBZ is highly persistent through physical, chemical and biologically process. Due to all these characteristics, it is currently considered a marker of environmental pollution.

Previous works have reported CBZ biodegradation with microalgae [23], strains isolated from activated sludge of a municipal WWTP [24], microbial enzymes [25] and white rot fungi [26, 27] even with high rates of CBZ biodegradation (80% in days or hours) [28, 29]. However, these high rates on recalcitrand PPCPS are often due to methodological errors in the procedure of extraction which overestimate the CBZ biodegradation. Therefore, the main goal of the current work was to obtain a microbial consortium with an efficient capacity to degrade CBZ. To achieve this aim, a serial passages experiment was conducted with a wood rot microbial consortium BOS08 [30] and a logistic growth model developed taking into account the interaction between the microbial consortium and the CBZ degradation.

Materials and Methods

Chemicals and Media

Carbamazepine (CBZ), purchase from Sigma-Aldrich, were prepared in a stock solution in water mili-Q, methanol and acetic acid (94.5:5:0.5; v:v:v) to obtain a final concentration of 200 mg l^{-1}. To calculate the saturation concentration in water, increasing additions of CBZ were added to miliQ-water till the precipitate was observed. From there, 3 ml were filtered to remove the precipitated CBZ and was analyzed by HPLC. The results obtained showed that the solubility of CBZ in water was 106.06 mg l^{-1}. Busnell Haas Broth medium (BHB) purchase from Sigma-Aldrich was used as culture media.

Experimental Design and Treatments Conditions

The microbial consortium BOS08 was extracted from dead wood in a Atlantic forest (Parque Natural de Fragas do Eume, Galician, Spain 43° 41.75′ N, 8° 6.83′ O) with PAHs-degrading capacity acquired once exposed to those pollutants [30]. Since, the consortium BOS08 did not show previous CBZ degradation capacity a serial passages experiment was conducted during 12 months with CBZ as the sole source of carbon. The idea has been to enrich the microbial consortium with strains having the metabolic capacity to degrade CBZ

A solution (A), was prepared with 900 ml of BHB with 100 ml of stock solution of CBZ (final concentration of 17 mg l^{-1}) and microbial consortium BOS08 (final bacteria concentration of 8.6×10^7 cell ml^{-1}). The solution (A) was distributed in 3 Erlenmeyer flasks of 250 ml (100 ml each replicate) to evaluate the microbial growth evolution.

To analyze the CBZ biodegradation rate and kinetics, 300 ml of solution (A) was distributed in 60 glass flasks (5 ml each replicate). To analyze the abiotic CBZ degradation rate, 50 ml of solution B (90 ml of BHB + 10 ml of stock solution of CBZ) was distributed in 9 glass flasks (5 ml each replicate). All samples were incubated in an orbital shaker at 200 rpm and at room temperature (21 °C).

Microbial Growth Evolution, Identification of the Microbial Consortium BOS08

At each collecting time (see Table 2 in the Supplementary Materials section) 2 ml of each Erlenmeyer flask were collected to measure bacterial density during CBZ degrading process. Bacterial concentration was estimated by changes in the absorbance of the culture media at 600 nm using a Spectronic Genesys spectrophotometer. One of the aliquots was centrifugated at 13,000 rpm during 1 min and used as blank. At initial (0 h), intermediate (77 h) and final times (579 h), 5 ml of each Erlenmyer flask were collected for the subsequent identification of microorganisms through massive sequencing (Illumina).

Bacterial Identification of the Consortium BOS08

The BOS08 consortium has been previously exposed to PAHs during long periods of time. As a result, there has confirmed that fungal DNA and fungal activity were negligible in comparison to bacteria [30]. For this reason, the microbial identification through massive sequencing has been focused solely within bacteria. Total DNA was extracted using Kit UltraClean Microbial DNA Isolation (Mobio, Inc., Solana Beach, California, United States) to perform the molecular identification of prokaryotes growing at the different treatments. DNA concentration of samples was determined using Quant-IT PicoGreen reagent (Thermo Fischer) and DNA samples (about 3 ng) were used to amplify the V3–V4 region of 16 S rRNA gene [31]. PCR products (approx. 450 pb) included extension tails (CS1, CS2) which allowed sample barcoding and the addition of specific Illumina sequences in a second low-cycle number PCR. Individual amplicon libraries were analyzed using a Bioanalyzer 2100 (Agilent) and a pool of samples was made in equimolar amounts. The pool was further cleaned, quantified and the exact concentration estimated by real time PCR (Kapa Biosystems). Finally, DNA samples were sequenced on an Illumina MiSeq Instrument under a 2 × 300 protocol. Sequencing runs were performed using the following specific sequencing primers, according to original Fluidigm protocols:

CS1 ACACTGACGACATGGTTCTACA for read 1
CS2 TACGGTAGCAGAGACTTGGTCT for read 2

After sequencing, reads were quality filtered according to Illumina standard values, demultiplexed and fastq files were prepared. For 16S sequencing, reads were mapped against the GreenGenes database using current applications of Base Space (metagenomics 16S Illumina).

CBZ and Intermediate Metabolites Extraction

At each collecting time (see Table 2 in Supplementary materials section) three replicates were analyzed to identify the CBZ concentration by solid phase extraction (SPE) and subsequent quantification by high performance liquid chromatography (HPLC). The intermediate metabolites, formed during the degradation of the CBZ, were identified by high performance liquid chromatography coupled to mass spectrometry (HPLC-MS). The content of CBZ present at each experimental time was extracted by SPE following procedure described in [32] using Strata C18-E cartridges (500 mg absorbent, Phenomenex). To test the efficiency of the CBZ extraction, 10 samples (2 replicates) with different concentrations of CBZ (from 10 to 20 mg l^{-1}) were extracted by two different methods (one or two washing steps; see below). Results showed the 2nd washing step improved significantly the CBZ extraction in a 15 % (the t-test values were $t = -9.6; df = 10; p > 0.001$). Therefore, the procedure followed in our work was the optimized one. The cartridges were preconditioned with 5 ml of filtrated methanol and with 5 ml of milli-Q autoclaved water. First washing step: samples (5 ml) were centrifuged at 13,000 rpm for 10 min and supernatant passed through the cartridge. The cartridges were dried for 30 min in vacuum and then 30 min in stove at 50 °C. Second washing step:the CBZ attached to the microbial pellet was washed with methanol (2 ml) and centrifugated at 13,000 rpm for 10 min. The compounds absorbed by the cartridge were eluted with 7 ml of methanol plus the previous supernatant (2 ml). The second washing step significantly improved the CBZ extraction by 15 % (the t test values were $t = 9.6; df = 10; p > 0.001$). Therefore, as a result of this improvement the second washing step was included in all sample extractions reducing the overestimation of the consortium biodegradation rates. The total 9 ml were collected in a glass vial to be evaporated in the extraction hood. The dried extracts were resuspended with 1 ml of phase A (milli-Q water:methanol:acetic acid; 94.5:5:0.5; v:v:v; pH 2.8) ready for analysis in HPLC.

Modelling the Kinetics of the BOS08-CBZ Mixture

In this section we develop a model which takes into account the interaction between the consortium and the carbamazepine degradation. Models in general [33] can be divided into deterministics and stochastics. Here we will take a deterministic approach. Many of the models

found in the literature predicts the lag phase in a biological system to a reasonable accuracy, but either they do not take into account the interaction between the components in a reciprocal way [34], or they assume a general form which is very difficult to interpretate from first principles [35] after fitting the data. This difficulty of interpreting the terms is extensible to deep learning or machine learning methods. In order to overcome these limitations, we have proposed a model based on first principles which takes into account how the consortium interact with the presence of carbamzepine explicitly and justified the biological origin of each terms.

To model the kinetics of the bacteria-carbamazepine mixture, we denote the density of carbamazepine by C and the bacteria concentration by B and write the dynamics as

$$\frac{dC}{dt} = f_1(B, C), \quad C(0) = C_0, \tag{1}$$

$$\frac{dB}{dt} = f_2(B, C), \quad B(0) = B_0. \tag{2}$$

The constants C_0, B_0 are the initial concentrations, and f_1 and f_2 the functions to determine.

We make the following choices for f_1 and f_2. For the bacteria growth rate function f_2, a logistic term proportional to $B(\beta_2 - B)$ is included to model how growth slows down when microbial density becomes so large that resources disponibility become limited. The interaction with CBZ is taken into account by a term proportional to $C(B - \beta_1)$ which considers that bacteria concentration needs to reach a minimum concentration before starting the exponential CBZ degradation phase. For the rate of carbamazepine consumption f_1 we make the assumption a la Monod that any homogeneous interacting term is proportional to the corresponding bacterial rate term but opposite in sign [36]. So there is a term proportional to $-C(B - \alpha_1)$. Note that by mass conservation the independent parameter α_1 should be closer or equal to β_1 if the degradation of CBZ is due to the direct bacteria action and our hypothesis is right. Those terms can be seen as a second order approximation to the model used by [35].

CBZ did not show any abiotic degradation or any significant endogen metabolism (see Fig. 4 in Supplementary materials) therefore, we will not include more terms in the model. We remark that adding extra terms in the model makes a better fitting for a particular set of data but it is not biologically justified.

Thus, the chosen functional forms are

$$\begin{aligned} f_1(B, C) &= -\alpha_0 C(B - \alpha_1) \\ f_2(B, C) &= \beta_0[C(B - \beta_1) + B(\beta_2 - B)]. \end{aligned} \tag{3}$$

If we integrate those equations in time, we get

$$C(t) = \int_0^t f_1(B, C)\, dt + B(0), \tag{4}$$

$$B(t) = \int_0^t f_2(B, C)\, dt + C(0). \tag{5}$$

From the experiments, we have $B_{exp}(t), C_{exp}(t)$ for a certain interval of time $t \in [0, T]$, so the task is to solve an inverse problem for ordinary differential equations in order to find the coefficients of the functions f_1, f_2. This problem can be approached using the Piccard contraction mapping technique [37]. We take the square difference between our model solution and the experimental one and integrate on the interval

$$\begin{aligned} \Delta_1^2 &= \int_0^T |B_{exp}(t) - B(t)|^2\, dt, \\ \Delta_2^2 &= \int_0^T |C_{exp}(t) - C(t)|^2\, dt. \end{aligned} \tag{6}$$

Minimizing Δ_1^2 and Δ_2^2 the coefficients of the model $\alpha_0, \alpha_1, \beta_0, \beta_1, \beta_2$ are found.

An important remark about the model should be done before leaving this section. The fitting could be made more accurate by adding extra terms into the model. However, that is not justified from first principles, and we warn against blindly using commercial software which usually fits the data to a generic quadratic expressions without any qualitative analysis [38].

Results and Discussion

The experimental data and the theoretical curves obtained from the fitting of the model are plotted in the Figs. 1 and 2. The fitted values for the coefficients of the model are in Table 1. Contrary to [39] and as in [24] nutritive sources such as glucose or yeast extract were not added in order to avoid nutrient depletion before CBZ biodegradation.

The consortium BOS08 begins in a latent phase of 100 h (Fig. 1) which is the observed period in which there is no CBZ degradation (Fig. 2). In agreement with our hypothesis the fitted values of α_1 and β_1 are very close (Table 1), so bacterial growth coincides with CBZ degradation. In the model, due to the term proportional to $C(B - \alpha_1)$ the

Table 1 The parameters model obtained by fitting the kinetic equations (3) with the experimental data

f_1		f_2	
C_0	1.6427	B_0	0.5955
α_0	0.3092	β_0	2.3807
α_1	0.5387	β_1	0.4953
		β_2	0.3426

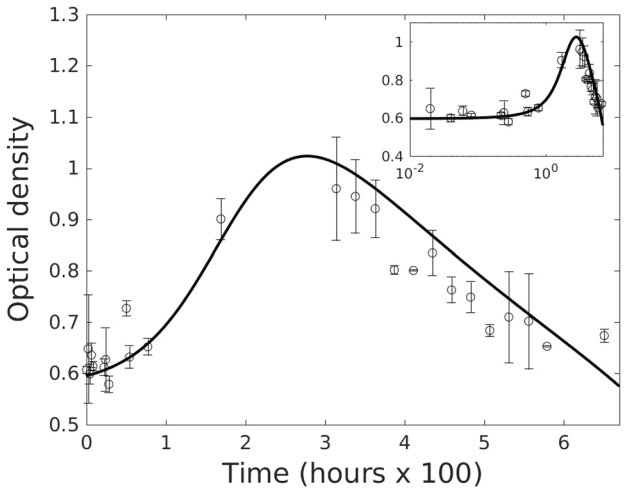

Fig. 1 Bacterias concentration versus time

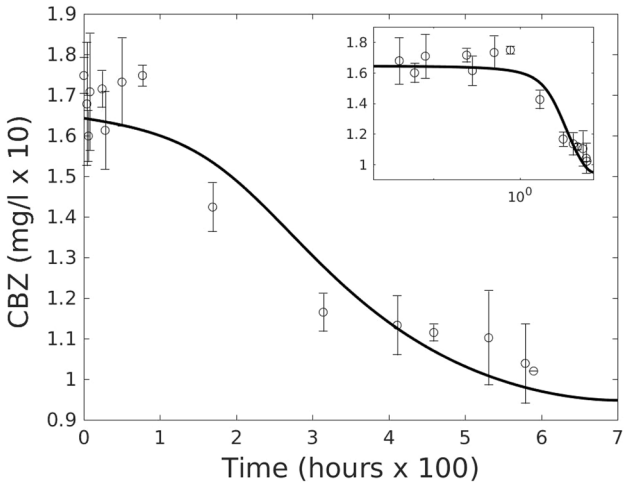

Fig. 2 Carbamazepine concentration versus time

bacteria concentration needs to reach a minimum concentration before the exponential CBZ degradation phase starts. Our results confirm previous findings [39, 40] that inoculum size has an important effect on recalcitrant biodegradation processes. The following phase (exponential phase) showed the highest CBZ degradation rate ($2.1 \times 10^{-3} \pm 4.2 \times 10^{-4}$ h^{-1}) and last approximately for another 100 h (see Fig. 1).

It is unlikely that all microbial populations within the consortium are able to degrade the recalcitrant components such as CBZ. However, this microbial consortium has been growing during serial transfer experiments with CBZ as the only carbon source. As a result, it has been forcing a selective pressure on the microbial consortium to allow the enrichment and isolation of bacterial strains with the ability to degrade CBZ. Therefore, the exponential phase suggests the evidence for this genomic evolution and consortium adaptation to these environmental conditions [41, 42]. How has been possible to acquire this adaptation? These microorganisms have been collected from a wood rot habitat where lignin is the most abundant organic carbon component. Lignin degrading microorganisms have oxidative, peroxidases and laccases enzymes with an important degradation role [43, 44]. These enzymes present a high oxidizing capacity and a low specificity to degrade other aromatic compounds [45] such as CBZ. Most probably, these constitutive enzyme systems are also involved in the degradation of CBZ demonstrated in *Streptomyces* [46]. According to the logistic term B (2 B) in the model, when microbial concentration is high and reaches a maxima, the environment becomes unfavorable being crowded and nutritionally limited [47–49]. Then the bacteria would suffer serious physiological stress and initiate the stationary phase. This phase lasted for 100 h and although, there was zero net growth, CBZ degradation was still observed (Fig. 2).

Cell concentration and other factors can regulate gene expression and therefore, bacterial metabolism by quorum sensing [50]. Indeed, as stated above, the term $C(B - \alpha_1)$ models the CBZ degradation regulated by cell concentration. When the microbial concentration reached the threshold, CBZ degradation was inhibited and the final phase of the curve started. This death phase lasted during 300 h.

Our extraction and detection methods provide an accurate CBZ degradation percentage avoiding an overestimation of the real biodegradation rate. The amount of CBZ degraded by the consortium BOS08 during the exponential and stationary phases (8 days) was approximately 40% in agreement with [39]. To optimize the CBZ biodegradation process, the latent phase should be reduced by increasing the initial inoculum size with a selection of the active dominant strains. Also, the addition of nutritive sources to the strains with CBZ degradation capacity which can stimulate extracellular enzymes production [51] will increase the efficiency.

Consortium BOS08 did not use co-metabolism (transformation of a non-growth substrate, in the obligate presence of a growth substrate) which is a frequent process during the degradation of recalcitrant components. Most studies have evaluated the CBZ biodegradation not considering the degradation routes and therefore, the intermediate metabolites production [11, 27, 29, 35, 39]. Our results showed the presence of 10,11-dihydroxycarbamazepine (CBZ-DiOH) as the unique intermediate metabolite during CBZ biodegradation process. The main pathway of CBZ is the oxidation to 10,11-dihydro-10,11-epoxycarbamazepine (CBZ-EP) catalyzed by several cytochrome isoforms, followed by the hydration to CBZ-DiOH (reaction mediated by an epoxide hydrolase) and conjugation with glucuronide [52–54]. More than 30 metabolites during the CBZ degradation have been identified [55]. CBZ biodegradation process by the consortium BOS08 is optimal and effective considering

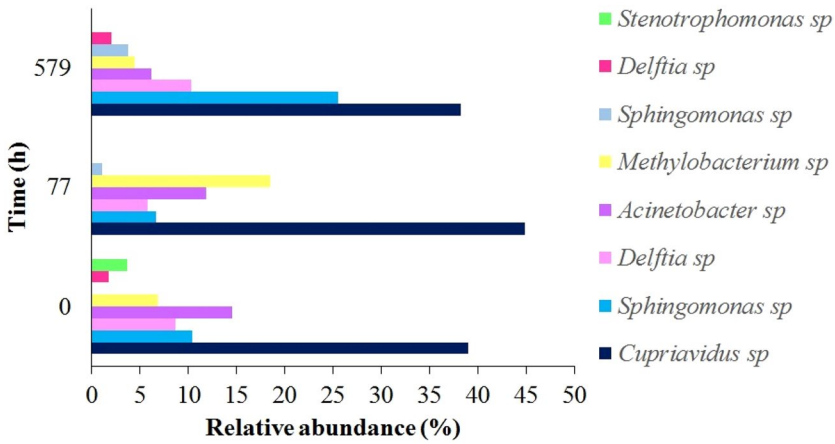

Fig. 3 Relative contribution of the most abundant genera within the consortium BOS08 during the CBZ degradation process at 0, 77 and 579 h

Table 2 Microbial evolution given by Optical density (600 nm) and CBZ degradation (mg l^{-1}) measured during 579 h

Hours	Optical density			CBZ (mg l^{-1})		
	R1	R2	R3	R1	R2	R3
0*	0.60	0.60	0.62	17.60	17.84	16.99
2	0.59	0.59	0.77			
4	0.61	0.61	0.58	18.08	15.11	17.16
6	0.61	0.65	0.65	16.38	15.27	16.32
8	0.60	0.62	0.62	16.90	18.60	15.75
22	0.60	0.62	0.62			
24	0.59	0.70	0.59	17.67	16.88	16.90
28	0.57	0.60	0.57	16.01	15.24	17.14
50	0.74	0.73	0.71	17.93	16.05	17.96
54	0.62	0.66	0.62			
77*	0.66	0.64				
169	0.89	0.87	0.95	14.92	13.79	14.00
314	1.01	1.02	0.84	11.98	11.32	
338	0.99	0.98	0.86			
363	0.97	0.93	0.86			
387		0.81	0.80			
411	0.80	0.80	0.80	11.60	11.89	10.50
435		0.87	0.80			
459		0.75	0.78	11.00	11.30	
483		0.73	0.77			
507		0.68	0.69			
531		0.65	0.77	10.20	11.84	
556		0.64	0.77			
579*	0.65	0.65	0.65	11.08	9.69	

Replicated samples $\mathbf{R_1}$, $\mathbf{R_2}$, $\mathbf{R_3}$. The * hours correspond to the collecting times for identifying microorganisms through massive sequencing (Illumina)

CBZ-DiOH is not pharmacologically active and toxic [32, 52], compared with CBZ-EP and other hydroxilated metabolites with biological activity and possible neurotoxic effects [56].

The consortium BOS08 has been originally extracted from wood rot in an Atlantic forest. However, it has been forced to several selective pressures during long periods of time to PAHs [30] and CBZ as the only carbon sources (present work). Previous results confirmed that fungal DNA and fungal activity were negligible in comparison to bacteria [30] under PAHs conditions. Indeed, bacteria produce enzymes to degrade more recalcitrant compounds later in wood decomposition [57] than fungi and contrary to previous results bacteria allocated between 2.3 and 25 times

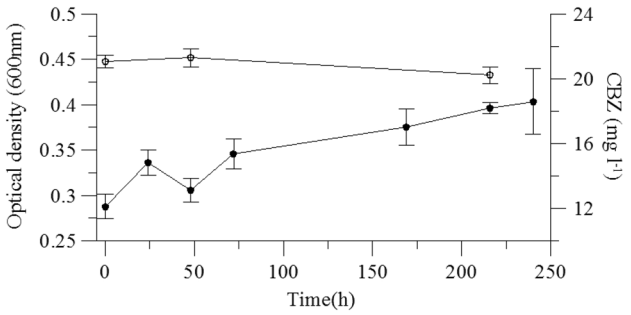

Fig. 4 Endogen metabolism of the microbial consortium BOS08 (full circles) and CBZ abiotic degradation (empty circles) versus time

more C to growth than did fungi in soil detrital food webs soils [58]. All these arguments somehow justify the identification of the bacteria, but always taking into account the importance of the entire microbial consortium including its interactions in the biodegradation of CBZ.

During all phases, there was a co-existence of five major genera from Proteobacteria phyla, comprising *Cupriavidus* sp. (38%), *Sphingomonas* sp. (29%), *Delftia* sp. (12%), *Acinetobacter* sp. (6%) and *Methylobacterium* sp. (4%). *Stenotrophomonas* sp. (4%) was only present during the latent phase (Fig. 3). Genera *Sphingomonas* and *Methylobacterium* are *α–Proteobacteria, Cupriavidus* and *Delftia* are *β–Proteobacteria* and *Stenotrophomonas* and *Acinetobacter* are *γ –Proteobacteria*. Most of them have been previously related with organic xenobiotics but not with CBZ biodegradation, with *Sphingomonas* being the exception [11, 59].

During PPCPs biodegradation, the dominance of *Proteobacteria phyla* has been confirmed, CBZ being one of the most recalcitrant compounds [60]. In *Cupriavidus* genera has been identified a large number of genes of xenobiotic degrading path-ways [59] but not with CBZ. Other PPCPs such as paracetamol have been degraded previously [61, 62] by *Delftia* and *Stenotrophomonas* genera as the sole carbon and energy source. *Methylobacterium* genera has not been previously described as having any CBZ biodegradation capabilities. This genera has a metylotrophic metabolism and mobile genes involved in the organic pollutants metabolism which makes them suitable for biodegradation processes [63]. So as CBZ is the only carbon source, our results suggest that the most dominant genera (see 3), including *Methylobacterium*, have been involved in CBZ degradation.

Conclusions

Experimental evolution systems allow the microbial consortium BOS08 an optimal and efficient capacity to biodegrade CBZ. The improved extraction method has provided an accurate CBZ depletion rate by BOS08 of $2.14 \pm 0.42 \times 10^{-3}$ h $^{-1}$. The consortium did not use co-metabolism to process CBZ and intermediate metabolites production were not pharmacologically active and toxic. The most dominant bacterial genera within the CBZ-degrader consortium were *Cupriavidus, Sphingomonas, Delftia, Acinetobacter, Methylobacterium* and *Sphingomonas*. None of them, with the exception of *Sphingomonas* have been previously related to CBZ degradation.

One outcome of the present work is the development of a mathematical model based on biological principles to predict the time evolution of the CBZ degradation process. The terms considered are of a logistic limited growth nature. The use of experimental data permits us to test the model and the new functional features acquired by the consortium. We remark that the inclusion of other terms is unnecessary and hardly justifiable from a biological point of view. Another important issue in this work is the optimization of the extraction protocols to avoid the overestimation in the biodegradation rates, mainly in recalcitrant components such as CBZ. Laboratory measurements combined with logistic growth models can be used to explain temporal structure and dynamics of natural microbial communities in order to predict the outcome of future experiments and improve the environment pollutants biodegradation. These type of procedures provide an eco-friendly tool for finding microbial consortia acting as relevant micro-factories for bioremediation applications.

Supplementary Materials

In Table 2 we collect the experimental data obtained in the laboratory. The endogen metabolism of BOS08 and the abiotic degradation of CBZ were also studied and are displayed in Fig. 4.

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Compliance with Ethical Standards

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

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