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ORIGINAL PAPER

Chemostat selection of a bacterial community able to degrade s-triazinic compounds: continuous simazine biodegradation in a multi-stage packed bed biofilm reactor

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Abstract Using a successive transfer method on mineral salt medium containing simazine, a microbial community enriched with microorganisms able to grow on simazine was obtained. Afterwards, using a continuous enrichment culture procedure, a bacterial community able to degrade simazine from an herbicide formulation was isolated from a chemostat. The continuous selector, fed with a mineral salt medium containing simazine and adjuvants present in the commercial herbicide formulation, was maintained in operation for 42 days. Following the lapse of this time, the cell count increased from 5×10^5 to 3×10^8 CFU mL^{-1}, and the simazine removal efficiency reached 96%. The chemostat's bacterial diversity was periodically evaluated by extracting the culture's bacterial DNA, amplifying their 16S rDNA fragments and analyzing them by thermal gradient gel electrophoresis. Finally, a stable bacterial consortium able to degrade simazine was selected. By PCR amplification, sequencing of bacterial 16S rDNA amplicons, and comparison with known sequences of 16S rDNA from the NCBI GenBank, eight bacterial strains were identified. The genera, *Ochrobactrum*, *Mycobacterium*, *Cellulomonas*, *Arthrobacter*, *Microbacterium*, *Rhizobium* and *Pseudomonas* have been reported as common degraders of triazinic herbicides. On the contrary, we were unable to find reports about the ability of the genus *Pseudonocardia* to degrade triazinic compounds. The selected bacterial community was attached to a porous support in a concurrently aerated four-stage packed-bed reactor fed with the herbicide. Highest overall simazine removal efficiencies η_{SZ} were obtained at overall dilution rates D below 0.284 h^{-1}. However, the multistage packed bed reactor could be operated at dilution rates as high as $D = 3.58$ h^{-1} with overall simazine removal volumetric rates $R_{v,SZ} = 19.6$ mg L^{-1} h^{-1}, and overall simazine removal specific rates $R_{X,SZ} = 13.48$ mg (mg cell protein)$^{-1}$ h^{-1}. Finally, the consortium's ability to degrade 2-chloro-4,6-diamino-1,3,5-triazine (CAAT), cyanuric acid and the herbicide atrazine, pure or mixed with simazine, was evaluated in fed batch processes.

Keywords s-Triazinic herbicides · Simazine biodegradation · Packed-bed reactor · Fed-batch process · Cyanuric acid

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Introduction

Simazine is a member of s-triazine herbicides, widely used to control broad-leaved weeds by inhibiting plant's photosystem II [32]. Due to their physicochemical properties, simazine is not appreciably adsorbed to minerals and potentially could be leached from clay and sandy soils. Herbicides can reach water reservoirs through spray drifts, run-off, soil erosion and leaching [18]; thus, simazine has been detected in surface water, lacustrine sediments and groundwater [23], exceeding the MCL of 4 μg L^{-1} established by USEPA [44].

Commonly used herbicides, such as simazine, have little acute toxicity for human beings; however, simazine is a compound identified by EPA as a reproductive toxicant because it affects various endocrine systems causing reproductive and developmental effects on aquatic species.

In human beings, simazine potentially causes adverse outcomes if exposure occurs during critical stages of human development [4, 41]. As an endocrine disrupting chemical (EDC), simazine has the possibility to exacerbate infectious diseases because EDCs disturb the human immune system by interfering with endocrine balance and inhibiting macrophage function. Macrophages are activated by microbial components such as lipopolysaccharides (LPS), and simazine is a known inhibitor of LPS-induced activation of macrophages [19, 20]. Usually, EDCs are ingested into the body through food, and since most EDCs are highly lipophilic and minimally degradable, they tend to accumulate in the body via the trophic chain. In Western Europe, simazine has been found widely distributed in grass, cow's milk, cereals, eggs and fruits, ranging from 2.32 to 387 $\mu g\ g^{-1}$ [5].

Conventional drinking water treatment processes [33], with the exception of activated carbon that is partially effective in removing the triazinic compounds [22], do not readily remove simazine and other slow reacting chloro-s-triazine compounds. Because of the stability of the s-triazine ring toward oxidation, complete mineralization could not be attained by ozonation, sonolysis, photolysis, or photocatalysis [1, 16, 27, 29]. Even, by using advanced oxidation processes, complete decontamination of chloro-s-triazine compounds could not be achieved [11].

Although not always possible, simazine can be completely degraded by microbial action in aerobic conditions. There are reports about simazine biodegradation by strains of algae [15], bacteria [12, 40], yeasts [35], fungi [14, 24], or mixed microbial communities [17, 25].

Processes for remediation of s-triazine contaminated soils are relatively frequent but reports on surface waters or groundwater decontamination are less common. However, some oxidation processes for remediation of water contaminated with s-triazines have been reported [8, 21, 31, 34]. Due to environmental concerns associated with the accumulation of pesticides in water supplies, there is a great need to develop safe, convenient, and economically feasible methods for pesticide removal [47]. Constructed wetlands are commonly used to treat polluted water. Although some pesticides could be effectively eliminated (>80%); others, such as simazine, are poorly removed ($\approx$20%) [6, 30], and in some cases s-triazine compounds are just transformed to their hydroxylated species [6]. In addition, in large water tropical microcosms, chloro-s-triazine herbicides such as atrazine and simazine have been identified as persistent pesticides ($DT_{50} \geq 47$ days), when compared with others that usually pollute tropical aquatic environments [26].

The full extent of the toxicity of pesticides to aquatic life is not known. In addition, very little is known about the toxicity and occurrence of pesticide breakdown byproducts, or about the non-pesticidal additives (co-formulants and adjuvants) used in pesticide formulations [10].

Some difficulties to decontaminate water polluted with chloro-s-triazine compounds exist. Due to high nitrogen to carbon ratio in the simazine molecule, their biodegradation often requires the addition of assimilable carbon sources to compensate the C/N unbalance, improving the rate of simazine degradation [24]. For agrochemical use, a liquid simazine formulation contains compounds such as ethylene glycol, polyglycol ether, alkyl ether sulfates, ethoxylated fatty alcohols or sulfosuccinic monoesters as surfactants [2, 3]. These adjuvants could be used as assimilable carbon sources, balancing the C/N ratio. Although a pure culture of a microbial strain would be able to metabolize a mixture of carbon sources such as that described above, a mixed microbial community would be more reliable to metabolize mixed substrates. Moreover, although adjuvants are typically categorized as "inert" compounds, many of them can produce wide-ranging effects on physiological and metabolic processes within plants, animals, and/or microorganisms [36, 37]; therefore, their removal from water is also of concern. Because of the contamination levels reached in the sources of primary water, mainly aquifers, the boundaries between "water" and "wastewater" treatment are already beginning to fade, particularly in underdeveloped countries with limited and polluted water resources. The elevated costs and time demanded to remediate a contaminated aquifer could be prohibitive to many of our countries. Hence, as an alternative to quarantine and ultimate remediation of contaminated sites, the application of processes for water decontamination, once extracted from a polluted aquifer, seems reasonable. For this reason, the selection of a bacterial community able to degrade triazinic compounds and its application to a process involving the degradation of the herbicide simazine in a multistage packed bed biofilm reactor has been developed in this work. In parallel, the consortium's ability to degrade simazine, atrazine and the metabolic byproducts, 2-chloro-4,6-diamino-1,3,5-triazine (CAAT) and cyanuric acid, was determined in fed-batch processes.

Materials and methods

Chemicals

All components used in culture media were obtained from Merck (Darmstadt, Germany). Aqueous simazine suspension concentrate (Simanex with 50% of active product) and simazine (98% purity) as a standard for HPLC were acquired from Agan Chemical Manufacturers, Ltd., Israel, and ChemService Co. USA, respectively. Atrazine (analytical standard), cyanuric acid (98% purity) and 2-chloro-4,6-diamino-1,3,5-triazine (95% purity), were acquired

from Sigma-Aldrich, USA. The solvents used for HPLC were purchased from J.T. Baker (USA).

Microorganisms

Using a successive transfer method on mineral salt medium containing simazine, a microbial community enriched with microorganisms able to grow on a minimal medium containing simazine was obtained from corn-cultivated soils (Tlahuac, D.F., México). Afterwards, using a continuous enrichment culture procedure, a mixed bacterial culture able to degrade simazine from an herbicide formulation was isolated from a chemostat, acting as a continuous selector. The chemostat was originally seeded with the enriched microbial community previously obtained. The chemostat continuously fed with a mineral salt medium containing simazine along with adjuvants present in the commercial herbicide formulation, was kept in operation for 42 days. Chemostat was operated at a dilution rate of $0.012 \pm 0.001\ h^{-1}$, at room temperature and pH 6.9 ± 0.2. Simazine concentration was routinely evaluated by spectrophotometric scanning of filtered samples (200–330 nm in a Beckman DU spectrophotometer). Results were periodically verified by HPLC. By extracting the culture's bacterial DNA (UltraClean soil DNA kit, Mo Bio Laboratories Inc. CA, USA), amplifying their 16S rDNA fragments [9], and analyzing them by thermal gradient gel electrophoresis [9], the bacterial diversity was periodically evaluated. Finally, a stable bacterial consortium able to degrade simazine was selected. Their components were isolated and identified by PCR amplification and sequencing of their 16S rDNA amplicons. This bacterial consortium was afterwards used in a multistage packed bed biofilm reactor.

Mineral salts (MS) medium

The media composition (in $g\ L^{-1}$) was: K_2HPO_4, 0.4; KH_2PO_4, 0.1; $MgSO_4{\cdot}7H_2O$, 0.10; NaCl 0.1; $CaCl_2$, 0.02. Five milliliters of a solution containing microelements was added to obtain a final concentration (in $mg\ L^{-1}$) of: $FeSO_4{\cdot}7H_2O$, 2.75; $ZnSO_4{\cdot}7H_2O$, 1.15; $MnSO_4{\cdot}7H_2O$, 0.33; $CoCl_2{\cdot}6H_2O$, 0.24; $CuSO_4{\cdot}5H_2O$, 0.24; $Na_2MoO_4{\cdot}2H_2O$, 0.17. As nitrogen and main carbon source, 100 $\mu L\ L^{-1}$ of aqueous simazine concentrate were added, reaching a simazine concentration of 50 $mg\ L^{-1}$. Microorganisms were maintained in agar slants with MS medium supplemented with aqueous simazine concentrate (50 mg simazine L^{-1}).

Identification of consortium's bacterial strains

Culturable bacterial strains showing morphologic differences in agar plates of MS medium were isolated. DNA from pure strains was extracted, and by PCR amplification, sequencing, and comparison of their 16S rDNA amplicons with the NCBI GenBank, the isolated strains were identified. For 16S rDNA amplification, four primers were used: FD1 [46], 8FLP, 13B, and 1492-RPL [39].

Determination of bacterial diversity by PCR-TGGE

16S rDNA fragments of about 500 bp were obtained by PCR amplification of extracted bacterial DNA, using U-968(GC) and L-1401 primers [13]. Amplicons were analyzed by thermal gradient gel electrophoresis (TGGE System Controller, Biometra, Germany).

Packed bed biofilm reactor

All experiments were carried out at room temperature in a laboratory multistage packed bed column (Fig. 1), composed of four tightly assembled glass-stages. A sintered glass plate with pore diameter 40–100 μm (Heraeus

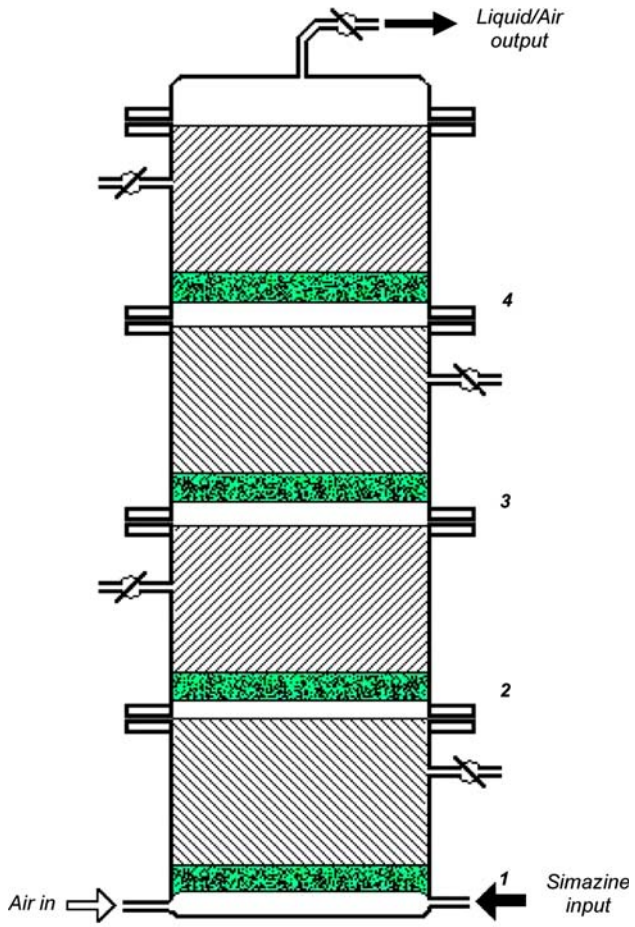

Fig. 1 Multistage packed bed reactor (MS-PBR). Stages *1–4* separated by sintered glass plates

Quarzglas GmbH & Co., Germany) was joined to the bottom at each stage. At the top of the column, an expansion head with a gas–liquid outlet was located. The column's support had entrances for liquid and air. Each stage had two sampling ports, packed with small fragments of porous volcanic stone. Once assembled, the total capacity (V_T) of the column was 636 mL, dry weight of porous support was 0.489 Kg and drained liquid volume V_L was 310 mL. The dimensions of the multistage packed bed reactor are shown in Table 1.

Packed bed biofilm support

Tezontle is an extrusive, usually dark grayish rock accumulated in the Trans-Mexican Volcanic Belt by volcanic eruptions. This material is broadly distributed in central México and is widely used in construction industry. The nonuniform rough particles of tezontle have a porous microstructure; consequently, the fluid in their interior is considered stagnant and intra-particular mass transfer occurs by diffusion. The equivalent diameter d_p of volcanic stone particles was 5.5 ± 1.04 mm; the apparent density of the packed bed ρ_{pb} was 0.27 Kg m^{-3}, and their void volume V_v was 62%.

Microbial cultivation in the multistage packed bed biofilm reactor (MS-PBR)

To evaluate the adsorption of simazine to porous support, a continuous abiotic test was conducted. MS-PBR was loaded on MS medium containing simazine (9.6 ± 0.7 mg L^{-1}). An airflow rate Q_{AIR} of 0.25 ± 0.02 L min^{-1} and a MS medium flow rate of 20 ± 1.2 mL h^{-1} were maintained. Simazine concentration was periodically determined in outflowing medium samples. Once the abiotic test concluded, the reactor's feeding was stopped. The flooded reactor was then inoculated with a cell suspension of the mixed microbial community, beginning a 120 h batch biodegradation process. Then, the reactor was operated in continuous regime using a peristaltic pump (Masterflex, Cole Parmer, USA), feeding it with MS medium containing simazine (9.6 ± 0.7 mg L^{-1}) as the sole nitrogen and carbon source. Overall dilution rates D varied from 0.0645 to 3.587 h^{-1}. The liquid stream flowing from the MS-PBR was sampled, and the simazine concentration (s) and As_{600} were determined. The overall dilution rate was maintained until s and As_{600} values remained stable (±5%), meaning that a steady state was reached. It was considered that the corresponding period to reach a steady state was at least five hydraulic retention times ($\theta_R = V_L/F$).

Microbial cultivation in fed-batch process

To evaluate the consortium's ability to metabolize the triazinic compounds; simazine, atrazine and the metabolic intermediaries 2-chloro-4,6-diamino-1,3,5-triazine (CAAT) and cyanuric acid, the microbial consortium was initially batch cultivated in the mineral-salts medium for 12 h, using any of the aforesaid triazinic compounds as the only nitrogen, carbon and energy source. Then, part of the culture was drained to initiate the fed-batch process with an initial volume V_0 of 50 mL. At this time, the culture was fed with mineral-salts medium (10 mL h^{-1}) supplemented with simazine (10 mg L^{-1}), atrazine (10 mg L^{-1}), a mixture of herbicides (5 mg L^{-1}, each), 2-chloro-4,6-diamino-1,3,5-triazine (CAAT) (10 mg L^{-1}) or cyanuric acid (10 mg L^{-1}). Each fed-batch culture was periodically sampled to determine substrate concentration. Only to visualize and compare the actual degradation level of the xenobiotic compounds fed to the reactor, the theoretical accumulation of a substrate c in the fed-batch reactor was calculated as if the microbial consortium did not degrade it.

The global accumulation rate of c in a fed-batch reactor is expressed as:

$$\left[\frac{\mathrm{d}Vc}{\mathrm{d}t}\right]_{acc} = V\left[\frac{\mathrm{d}c}{\mathrm{d}t}\right] + c\left[\frac{\mathrm{d}V}{\mathrm{d}t}\right] = Fc_R - \left[\frac{\mathrm{d}Vc}{\mathrm{d}t}\right]_{deg} \quad (1)$$

If this equation is solved under the assumption that the degradation rate $[\mathrm{d}Vc/\mathrm{d}t]_{deg}$ is zero, it can be described the transient accumulation $c[t]$ of the compound in the system when the volume V expands at a rate $\mathrm{d}V/\mathrm{d}t = F$. In this

Table 1 Dimensions of the multistage packed bed reactor

Stage number	Internal diameter (mm)	Internal height (mm)	Stage volume (cm^3)	Liquid volume (cm^3)	Porous support volume (cm^3)
1	51	93	190.0	92.6	97.4
2	51	88	179.8	87.6	92.2
3	51	87	177.7	86.6	91.1
4	47	51	88.5	43.1	45.4
MS-PBR total volume (cm^3)			636	309.9	326.1

expression, c_R represents the compound concentration in the medium fed at a volumetric flow rate F.

Solving the balance Eq. (1) for the initial conditions V_0, c_0; $c[t]$ was obtained:

$$c[t] = \frac{V_0(c_R - c_0)}{V_0 + Ft} \quad (2)$$

The initial conditions of the fed-batch process at $t_0 = 0$ were: $V_0 = 0.05$ L, the initial concentration c_0 of the triazinic compound used in each experiment was the final concentration obtained at the end of the preceding batch culture.

Analytical methods

Cell concentration in liquid samples of the MS-PBR

Once a steady state was reached, cell concentration of liquid samples collected at the MS-PBR was turbidimetrically determined at 600 nm (Beckman DU Spectrophotometer). Also, the viable cell concentration was determined by plate cell counting (CFU mL^{-1}) in MS-Agar plates containing simazine (100 mg simazine L^{-1}).

Biomass immobilized in porous support

Attached viable-biomass was determined by plate cell counting [CFU (g porous dry support)$^{-1}$]. Once continuous culture runs were finished; packed-bed column stages were sampled. Cells were water extracted from volcanic stone samples with the aid of a Vortex agitator. Stone fragments were washed and rinsed until a clear extract was obtained; then, appropriated dilutions of the collected cell suspension were used for viable cell counting on nutrient-agar plates. Another aliquot was used for cell protein determination by Lowry method [28].

Concentration of triazinic compounds

From sample filtrates, simazine, atrazine, and CAAT were determined by column liquid chromatography using a Beckman HPLC System equipped with an Alltech Econosphere C18 reverse-phase column and a diode-array detector (UV 280 nm). A water–acetonitrile reversed phase gradient elution was used. The acetonitrile concentration varied linearly from 10 to 100%. The flow rate used was 1.0 mL min^{-1} [7]. For HPLC determination of cyanuric acid, an isocratic mobile phase of 5.0 mM sodium octan sulfonate on 0.05% H_3PO_4 was injected to an Altech Inertsil column at a flow rate of 1.0 mL min^{-1} [43].

Results and discussion

Microbial community selection in chemostat

Changes in viable cell count and simazine concentration occurring along 42 days of continuous selector operation are shown in Fig. 2. Viable cell mass increased from 5×10^5 to 3×10^8 CFU mL^{-1}, and the simazine concentration decreased from 7.5 to 0.31 mg L^{-1}, representing a removal efficiency of about 96%. At this time, DNA extracted from a cell package obtained by sample centrifugation was purified and used as template for PCR amplification of bacterial 16S rDNA.

Isolation and identification of bacterial constituents

By sequencing the bacterial 16S rDNA amplicons and comparison with known sequences of 16S rDNA from the NCBI GenBank, eight bacterial strains were identified (Fig. 3). Genera *Ochrobactrum* (GeneBank, accession number AM084042 with 98% homology), *Cellulomonas* (X83804, 97% homology), *Arthrobacter* (AY167856, 95% homology), *Microbacterium* (AM181504, 97% homology), *Rhizobium* (AM084043, 93% homology), *Mycobacterium* (AJ833917, 90% homology) and *Pseudomonas* (DQ303435, 98% homology) have been reported as common degraders of triazinic herbicides [38, 42, 45]. On the contrary, we were unable to find reports about the ability of the genus *Pseudonocardia* (DQ856329, 86% homology) to degrade triazinic compounds.

Growth of isolated bacterial strains on simazine

Using the streak plate method, the isolated bacterial strains were individually inoculated on MS agar plates containing simazine as the sole carbon and energy source. Growth was evident after incubating them for 96 h at 28°C. All the strains could grow on simazine, although *Pseudomonas* sp.

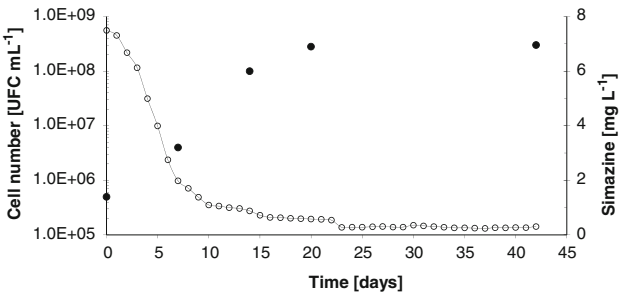

Fig. 2 Viable cell count and simazine concentration at the output of the continuous selector continuously operated at a dilution rate of 0.012 h^{-1}. Simazine (*open circle*) and cell plate count (*filled circle*) at MS-PBR's output

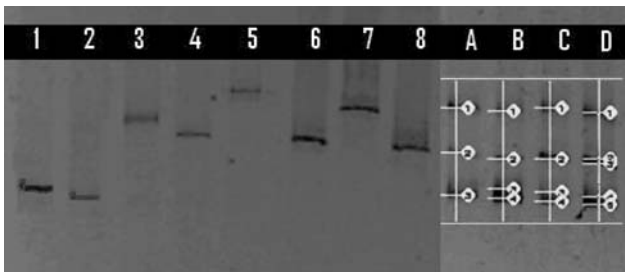

Fig. 3 Temperature gradient gel electrophoresis (TGGE) pattern of DNA fragments of about 500 bp, obtained by PCR-amplifying of DNA extracted from strains pertaining to the microbial community inoculated to the PBR (*Lanes 1–8*). *1 Mycobacterium ratisbonense*, *2 Cellulomonas cellasea*, *3 Pseudomonas sp*, *4 Arthrobacter globiformis*, *5 Rhizobium* sp, 6 *Ochrobactrum* sp, *7 Microbacterium hominis*, *8 Pseudonocardia* sp. The bands present on *lanes A–D* correspond to the strains detected at each column's stage after 332 days of PBR's operation. *A* Fourth stage, *B* third stage, *C* second stage, *D* first stage

and *Rhizobium* sp. showed poor growth. On the other hand, the bacterial community presented good growth after 24 h incubation, denoting that the concerted action of part, or all bacterial strains involved in the biodegradation of simazine or its metabolic byproducts remarkably increased the growth rate of the bacterial community.

Simazine biodegradation in a multistage packed bed biofilm reactor (MS-PBR)

To determine the simazine adsorption onto porous stone fragments, an abiotic test was run in the aerated MS-PBR. Along the 5-days test, it was observed that the outflowing simazine concentration represents about 96% of the inflowing simazine. Thus, the main part of herbicide removal should be caused by microbial action and not by support's adsorption or by air stripping. The selected bacterial community, once attached to porous volcanic stone fragments, was used in the concurrently aerated packed-bed bioreactor fed with a mineral salt medium containing simazine (9.65 ± 0.7 mg L^{-1}). The reactor operated along 332 days. Thirteen overall dilution rates D ranging from 0.065 to 3.62 h^{-1}, corresponding to overall hydraulic retention times varying from 15.38 to 0.28 h, respectively, were probed. Overall simazine removal efficiencies η_{SZ}, overall simazine removal volumetric rates $R_{v,SZ}$ and overall simazine removal specific rates $R_{X,SZ}$ are shown in Table 2 as a function of the dilution rates used in the multistage packed bed reactor. The MS-PBR was operated with loading rates $B_{V,SZ}$ as high as 34.5 mg L^{-1} h^{-1}, with maximum dilution rates of 3.62 h^{-1}.

Figure 3 shows the TGGE of DNA fragments of about 500 bp, obtained by PCR-amplification of DNA extracted from bacterial biofilm attached to the porous support of each column's stage. The bands observed on lanes A–D correspond to the strains detected by TGGE image analysis at each column's stage, after 332 days of reactor's operation. Lanes 1–8 correspond to the strains pertaining to the bacterial community isolated by chemostat selection, which were inoculated at the startup of the MS-PBR operation. At the end of the last run, only five strains were detected in support's attached-biofilm. Three were widespread in all reactors' stages (*Cellulomonas cellasea*, *Pseudonocardia*, and *Microbacterium hominis*), *Mycobacterium ratisbonensi* was present in the first three stages, but not in the fourth, and *Ochrobactrum sp* was only detected in the first stage. *Pseudomonas* sp., *Rhizobium* sp. and *Arthrobacter globiformis* do not prevail in the packed bed reactor.

Figure 4 shows variation in viable cells, simazine and cyanuric acid concentrations obtained at the distinct

Table 2 Overall behavior of the MSPBR. At all dilution rates probed, the simazine concentration was maintained at 9.6 ± 0.7 mg L^{-1}. The overall attached cell protein concentration was 1.4537 g (g support)$^{-1}$

Overall dilution rate D (h^{-1})	Volumetric removal rate $R_{V,SZ}$ (mg simazine L^{-1} h^{-1})	Specific removal rate $R_{X,SZ}$ [mg simazine (g cell protein)$^{-1}$ h^{-1}]	Removal efficiency η_{SZ} (%)
0.065	0.641	0.441	100.0
0.090	0.898	0.618	100.0
0.13	1.283	0.883	100.0
0.17	1.630	1.121	100.0
0.18	1.797	1.236	100.0
0.21	2.050	1.410	100.0
0.28	2.816	1.937	100.0
0.41	4.090	2.813	99.5
0.67	6.550	4.506	97.9
1.20	11.689	8.041	97.7
1.85	15.937	10.963	85.4
2.80	17.557	12.077	59.1
3.62	19.601	13.484	50.1

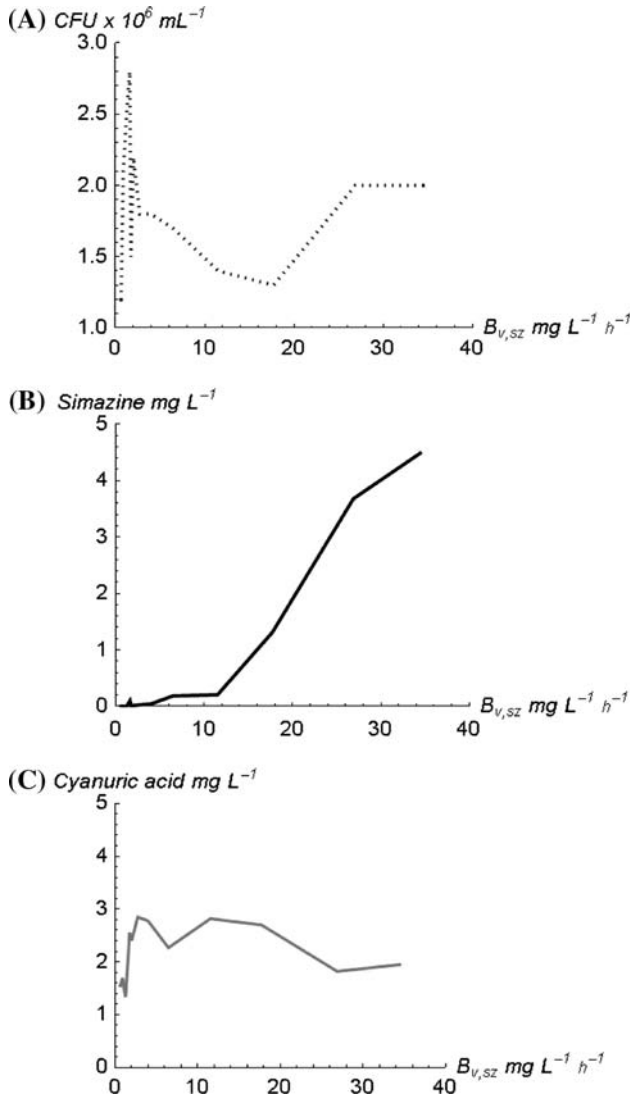

Fig. 4 Viable cells (**a**), simazine (**b**) and cyanuric acid (**c**) concentrations at the output of the MS-PBR continuously operated at varying volumetric loading rates $B_{v,sz}$

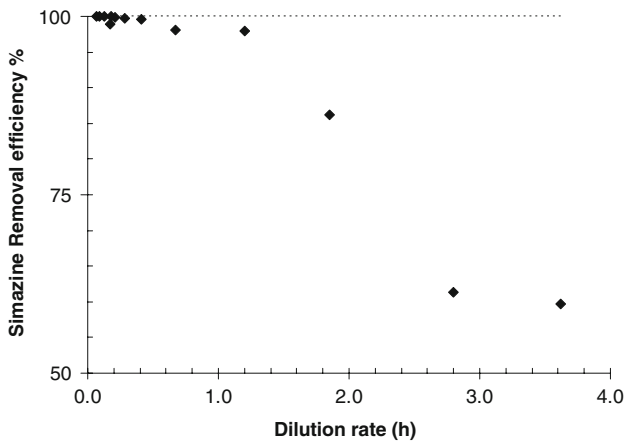

Fig. 5 Influence of overall hydraulic retention time (HRT) on simazine removal efficiencies obtained in a multistage packed bed reactor

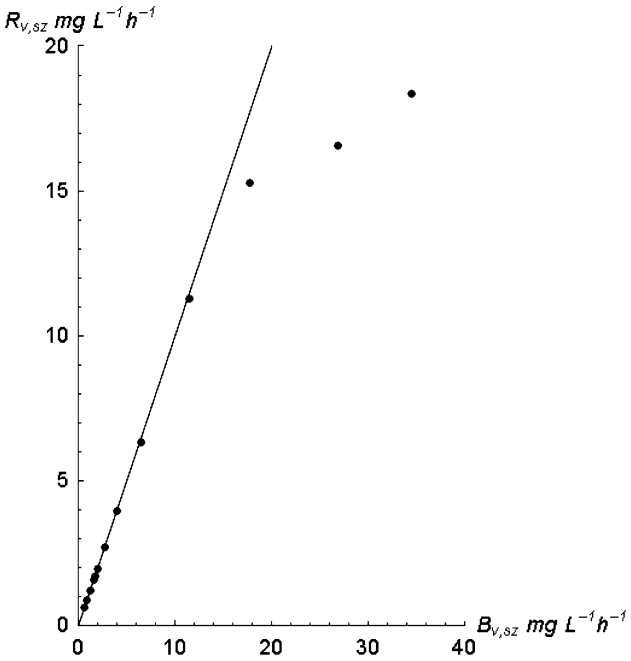

Fig. 6 Overall simazine removal rates $R_{V,SZ}$ obtained in the MSPBR at different simazine loading rates $B_{V,SZ}$. The values of $B_{V,SZ}$ and $R_{V,SZ}$ were calculated as: $B_{V,SZ} = F\,[s_o]/(\Sigma V_{stage})$; $R_{V,SZ} = F\,[s_o - s_{out}]/(\Sigma V_{stage})$

volumetric loading rates used. Small $B_{V,SZ}$ values means starvation conditions for the attached biofilm, presumptively in the last reactor stage. At the lowest simazine loading rates $B_{V,SZ}$ (less than 2 mg L^{-1} h^{-1}), and dilution rates D probed (less than 0.21 h^{-1}), an important release of attached cells (Fig. 4a), together with low simazine (Fig. 4b) and cyanuric acid (Fig. 4c) concentrations were observed. In spite of cell detachment, the highest values of η_{SZ} were obtained in those conditions. However, η_{SZ} values bigger than 97% could be reached at dilution rates as high as 1.2 h^{-1}, (Fig. 5). As observed in Fig. 6, beyond that $B_{V,SZ}$ value, the values of η_{SZ} decayed.

Once the MS-PBR operation was finished, the column was disassembled. Cell protein and cell number was determined in porous support. Figure 7 shows the cell and protein distribution at each reactor's stage.

Kinetic information about simazine biodegradation processes is scarce in literature. For a surface water treatment process used to extend the bed life of granular activated carbon (GAC), Feaking et al. [12] reported biodegradation efficiencies of 58 ± 8.2% for simazine adsorbed into GAC packed-bed (13.9 μg g^{-1} GAC) inoculated with *Rhodococcus rhodochrous*. We were unable to

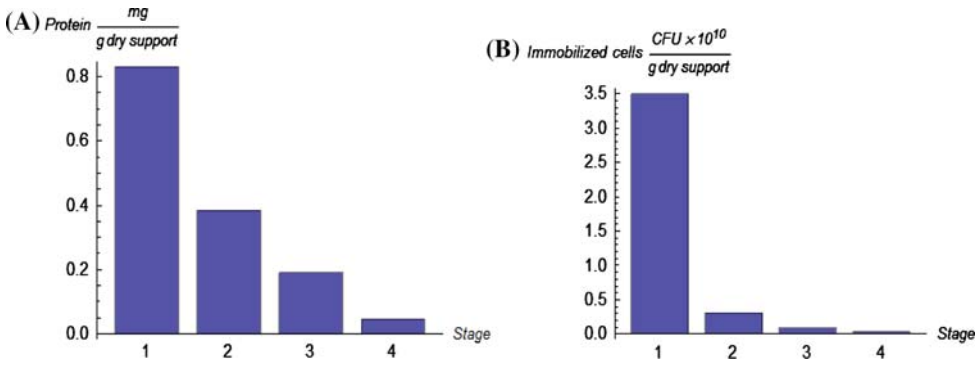

Fig. 7 Cells immobilized in porous support at each reactor's stage. Cell mass determined as protein (**a**), immobilized viable cells (**b**)

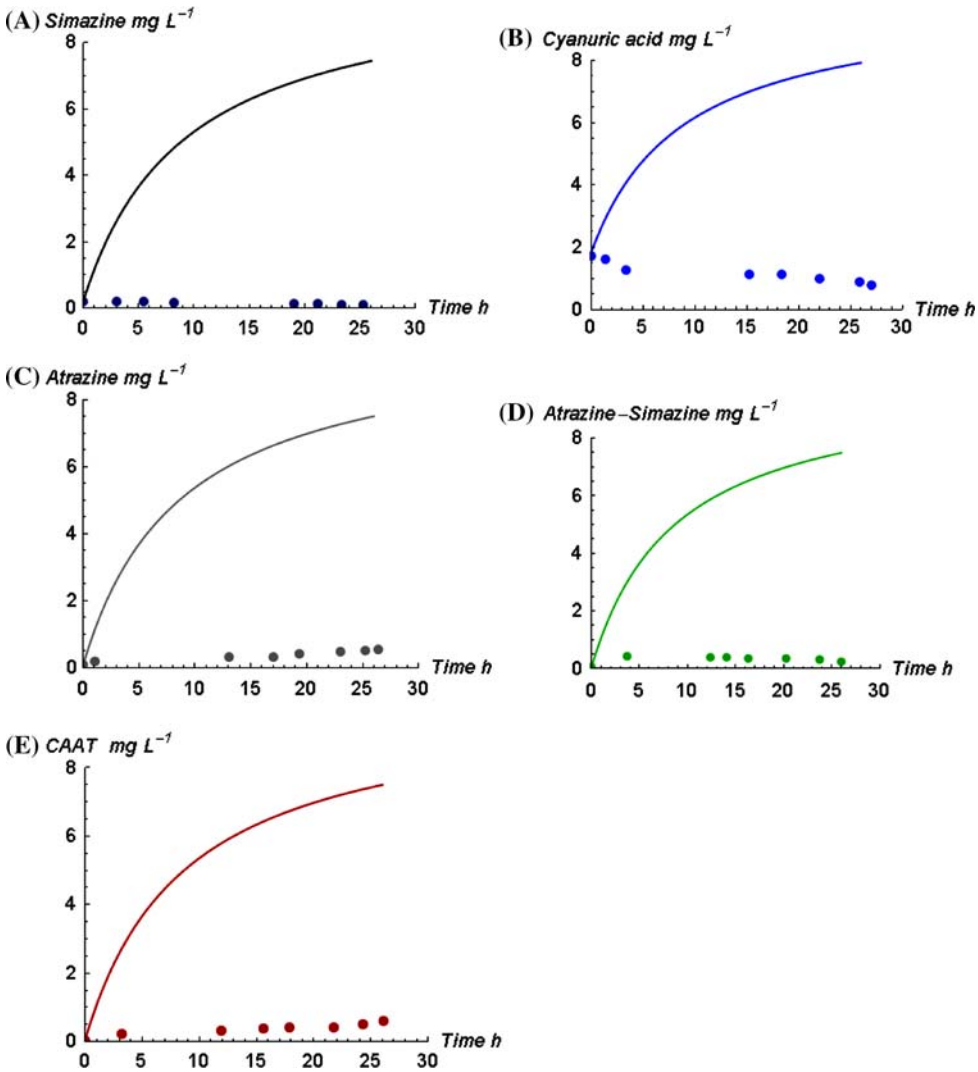

Fig. 8 Microbial consortium ability for degradation of s-triazinic compounds in fed-batch processes. *Solid lines* Theoretical accumulation of s-triazinic compounds, if cells were unable to degrade them. *Points* Experimental determination of triazinic compound concentration. **a** Simazine, **b** cyanuric acid, **c** Atrazine, **d** Mixture of atrazine–simazine [50:50]; and **e** 2,Chloro,4,6-diamine-s-triazine (CAAT)

find kinetic data related to volumetric or specific simazine removal rates, obtained in the biodegradation processes.

Consortium's ability to metabolize triazinic compounds in fed batch processes

In a fed-batch culture supplied with a constant substrate flow, microbial cell mass usually accumulates. In this reaction system, the volumetric loading rate R_{VFB} decreases linearly in accordance to:

$$R_{VFB}[t] = \frac{Fc_R}{V_0 + Ft} \quad (3)$$

At low substrate loading rates, the culture ultimately becomes substrate limited. This condition permits the metabolic byproducts, transiently accumulated, to be

degraded by the microbial consortium, improving the substrate removal efficiency. Consortium's ability to metabolize triazinic compounds using this culture system could be observed in Fig. 8. From the final values of V_f, and c_f, obtained at the end of each fed-batch culture ($t_f = 26$ h), the overall removal efficiencies η_{FB} were calculated with Eq. (4):

$$\eta_{FB} = \frac{V_0 c_0 - V_f c_f + F c_R t_f}{V_0 c_0 + F c_R t_f} \quad (4)$$

In any case, the removal efficiencies obtained were smaller than 90%. The higher efficiencies obtained in fed-batch cultures corresponded to simazine, 98.5%, and to the mixture of atrazine–simazine, 96.8%.

Conclusions

The classic microbiological method of chemostat enrichment was used for selecting microbial strains from a large diverse population by applying particular selective pressures such as substrate limitation by a particular nutrient source. In our case, the method was useful for selecting a microbial community constituted by eight bacterial strains involved in the biodegradation of simazine, atrazine, 2-chloro-4,6-diamino-1,3,5-triazine (CAAT) and cyanuric acid, using them as the sole nitrogen and carbon source, as was afterwards probed using suspended cell fed-batch cultures.

The MS-PBR was continuously operated at 13 dilution rates, ranging from 0.065 to 3.62 h^{-1}, for almost 8,000 h. The biofilm reactor could be operated with high simazine loading rates $B_{V,SZ}$, while elevated simazine removal efficiencies η_{SZ}, were obtained. However, cyanuric acid accumulation was always observed in the continuous system.

The fed-batch strategy used to evaluate the biodegradability of organic toxic compounds was convenient because low substrate concentrations was always maintained, avoiding their possible inhibitory effects, and favoring the biodegradation of intermediary metabolites that could be accumulated in the reactor. In all cases, substrate removal efficiencies higher than 90% were obtained for the compounds probed.

When large water bodies have to be treated to remove toxic or recalcitrant pollutants, most physicochemical and biological methods useful for industrial wastewater treatment become impractical or uneconomical due to the low pollutant concentrations found in groundwater or surface waters. Technologies that use adsorbent materials as granular activated carbon (GAC) are well accepted to get potable water from primary water sources. However, competition of organics reduces the adsorptive capacity for any individual compound, limiting the life of GAC filters by adsorbent saturation with natural organic matter and chemical pollutants. Another limitation is the flow diminution and filter's pressure drop caused by particle fragmentation and bed compacting. Bioprocesses designed to treat polluted water using microorganisms attached to GAC could solve the adsorbent saturation, but not the particle fragmentation, or the pressure drop caused by GAC compacting. The use of volcanic scoria as rigid support material for attached microbial communities could economically reduce the above-mentioned problems. Specially, when mixed contaminants are present in water, an adequate microbial population density can be supported for remediation of aquatic systems.

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