Water Air Soil Pollut (2018) 229: 120
https://doi.org/10.1007/s11270-018-3780-7

Biodegradability of Disinfectants in Surface Waters from Buenos Aires: Isolation of an Indigenous Strain Able to Degrade and Detoxify Benzalkonium Chloride

María Susana Fortunato · Sabina Baroni · Ana Julieta González · Jesús David Álvarez Roncancio · Mariana Papalia · Manuela Martinefsky · Valeria Trípodi · Estela Planes · Alfredo Gallego · Sonia Edith Korol

Received: 2 September 2017 / Accepted: 14 March 2018 / Published online: 21 March 2018

Abstract Biodegradability of chlorhexidine (CH), triclosan (TC), and benzalkonium chloride (CBA) has been tested in 18 surface water sampling points in the urban area of Buenos Aires. Sampling points were located in both the Reconquista and the Matanza-Riachuelo basins as well as in the La Plata River. High tolerance to the three disinfectants was found and indigenous strains capable of degrading CBA and TC were isolated. Neither tolerance nor biodegradation were correlated with sewage pollution. A strain that degrades CBA was identified as belonging to the genus *Pseudomonas* using the API20NE system and 16SRNA sequencing. In batch assays, the strain was capable of degrading 100, 200, and up to 500 mg L^{-1} of CBA in 10, 25, and 46 h respectively with specific growth rates (μ) of 0.56, 0.30, and 0.14 h^{-1}. The efficiency of the process was between 99.5–98.0% in terms of compound removal and between 93.8–89.1% in terms of chemical oxygen demand (COD). The detoxification of the compound as a result of the biodegradation was assessed using *Pseudokirchneriella subcapitata*, *Vibrio fischeri*, and *Lactuca sativa* as test organisms.

Keywords Benzalkonium chloride · Triclosan · Chlorhexidine · *Pseudomonas* · Biodegradation · Detoxification

M. S. Fortunato · S. Baroni · A. J. González · J. D. Álvarez Roncancio · A. Gallego (✉) · S. E. Korol
Facultad de Farmacia y Bioquímica, Cátedra de Salud Pública e Higiene Ambiental, Universidad de Buenos Aires, Junín 956 4° Piso (1113), Buenos Aires, Argentina
e-mail: agallego@ffyb.uba.ar

A. J. González · V. Trípodi
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

M. Papalia · V. Trípodi
Facultad de Farmacia y Bioquímica, Laboratorio de Resistencia Bacteriana, Cátedra de Microbiología, Universidad de Buenos Aires, Junín 956 8° Piso (1113), Buenos Aires, Argentina

M. Martinefsky · V. Trípodi
Facultad de Farmacia y Bioquímica, Departamento de Tecnología, Farmacéutica, Universidad de Buenos Aires, Junín 956 6° Piso (1113), Buenos Aires, Argentina

E. Planes
Centro de Química y Petroquímica (CEQUIPE), Instituto Nacional de Tecnología Industrial, Av. General Paz N° 5445 (1650), San Martín, Provincia de Buenos Aires, Argentina

1 Introduction

Emerging pollutants are defined as compounds that are not currently covered by existing water quality regulations, have not been studied before, and are thought to be potential threats to environmental ecosystems and human health and safety (La Farré et al. 2008). These compounds are usually found in the environment at very low concentrations, but due to their high biological activity they are equally of concern (Kümmerer 2004a). This is the case of disinfectants, a group considered of special importance for its potential environmental impact, since it could contribute to the selection

of resistant microorganisms. (Kümmerer 2001a; Hall-Stoodley et al. 2004). Exposure to sub-inhibitory biocide concentrations not only facilitates the evolution of resistance to the biocide involved, but also leads to co-resistance and cross-resistance to other antimicrobial agents such as antibiotics (Gaze et al. 2005; Ying 2006).

Within the disinfectants group there are compounds of special interest due to their production volume and characteristics such as: chlorhexidine, triclosan, and quaternary ammonium compounds (QACs). A chlorhexidine (CH) salt, digluconate, is primarily used in the formulation of disinfectants, cosmetics, and as a preservative for pharmaceutical products (Das et al. 2015). Triclosan (TC) is a nonionic, broad-spectrum antimicrobial used as an ingredient in disinfectants, soaps, detergents, toothpastes, mouthwashes, deodorants, and shampoos, and in addition to many other personal care, veterinary, industrial, and household products (Dann and Hontela 2011). QACs are currently the major class of cationic surfactants used as ingredients in fabric softeners, antistatics, disinfectants, biocides, detergents, phase transfer agents, and numerous personal care products (Tezel and Pavlostathis 2009; Ivanković and Hrenović 2010; Lara Martín et al. 2010). Benzalkonium chloride (BAC) is one of the most important QACs (Sütterlin et al. 2008a). BAC consists of a mixture of alkyl benzyl dimethyl ammonium chlorides with C8 to C18 alkyl groups. The most commonly encountered homologs are C12, C14, and C16 (Prince et al. 1999; Zhang et al. 2011).

Due to their extensive use, disinfectants and their derivatives are often present in substantial amounts in wastewater treatment plants (Zhang et al. 2015). BAC is one of the most frequently reported disinfectants worldwide. Several authors reported its occurrence in municipal wastewater at concentrations ranging from 1.1 to 36.6 μg L^{-1}; whereas in effluents from hospitals, laundries, and food industries the concentration levels reported varied between 0.9 μg L^{-1} and 6 mg L^{-1} (Kümmerer et al. 1997; Ferrer and Furlong 2001, Ding and Liao 2001; Martínez-Carballo et al., 2007a; Clara et al. 2007). Treatment plants are not usually designed to carry out an efficient degradation of such toxic and persistent compounds. Thus, a high proportion of disinfectants undergoes no alteration and can reach the aquatic environment. It should be noted that in many cases there is not even a properly functioning treatment plant; as a result, the presence of these compounds in waterways has been extensively documented (Jones et al. 2001, Kümmerer 2004b, Dynes et al. 2006, Sütterlin et al. 2008a, Dougherty et al. 2010, Dann and Hontela 2011, Zhang et al. 2015). Moreover, disinfectants can reach the terrestrial environment through the application of biosolids as soil amendment (Xia et al. 2005) Total concentrations ranging from 22 to 167 mg kg^{-1} have been measured for QACs in biosolids (Martínez Carballo et al., 2007b; Li et al. 2014).

In addition to their role in the spread of antibiotic resistance, many disinfectants have a direct toxic effect on aquatic organisms. CH shows a high toxicity to *Pseudokirchneriella subcapitata* and *Daphnia magna*, suggesting that this compound may cause adverse effects in aquatic ecosystems (Jesus et al. 2013). Also, TC and QACs are toxic to a lot of aquatic organisms including fish, daphnids, algae, bacteria, and rotifers (Zhang et al. 2015; Dann and Hontela 2011; Sütterlin et al. 2008b).

Since the input of these contaminants in the environment is continuous, dissipation is expected to be due to biodegradation, photolysis, and sorption, not dilution (Morrall et al. 2004). The three groups of compounds are poorly biodegradable and resistant to hydrolysis. The estimated half-life in water is 180 days for CH, 60 for TC, and 38 for BAC (EPA 2017). Removal by sorption to sediment and suspended material can be significant (La Farré et al. 2008), but it does not lead to the disappearance of the problem.

In order to minimize the dumping of these pollutants into waterways, attempts to optimize the operation of effluent treatment plants have been made, sometimes adding physicochemical treatments such as ozonation (Rosal et al. 2010) or by the photo-Fenton process (González et al. 2007). The use of bioaugmentation strategies, employing bacterial strains from natural sources capable of degrading these compounds, could also be an appropriate strategy to improve process efficiency (Fortunato et al. 2016). Much of the literature on disinfectant contamination is focused on improving the efficiency of municipal treatment plants to eliminate low concentrations resulting from the use of these compounds, which are undoubtedly the main source of contamination. However, it should be noted that point contamination with potentially high concentrations can occur as a consequence of the use of the compounds as raw material for the formulation of different products. In all cases, not only the degradation of the compounds, but also the absence of toxicity should be verified, since as a consequence of chemical or biological transformations even more toxic metabolites than the starting

products could be formed (Hernando et al. 2005; González et al. 2007).

Most of the results of environmental concentrations of emerging contaminants come from developed countries. In other regions, for example in Argentina, as a result of the difficulty in determining the expected low concentration of pollutants the situation remains unknown. Furthermore, studies on disinfectants biodegradability in surface waters in the region are likewise lacking.

The purposes of the present investigation were (a) to study the biodegradability of CH, TC, and BAC on a laboratory scale in surface waters from the urban area of Buenos Aires; (b) to isolate the microorganisms involved in the biodegradation process; (c) to employ the selected microorganisms in the batch treatment of synthetic wastewater contaminated with the disinfectants; and (d) to assess detoxification using standardized toxicity tests.

2 Materials and Methods

2.1 Chemicals

BAC (purity 98.5%) was supplied by Carlo Erba (Sabadell, Spain). TC and CH for production of medicinal preparations (purity 99%) was obtained from a local pharmaceutical company. Bromothymol blue was supplied by Chroma Gesselschaft (Stuttgart, Germany). Disinfectant stock solutions employed in biodegradation assays (10,000 mg L^{-1}) were prepared by dissolving the necessary amount of each disinfectant in water and sterilized by filtration. Phosphoric acid (85%) analytical grade was supplied by Mallinckrodt (New York, USA) and methanol was supplied by Sintorgan (Buenos Aires, Argentina). Ultrapure water was obtained by EASY pure RF equipment (Barnstead, Dudubuque, IA, USA). All solutions used for HPLC were filtered through a 0.45-μm nylon membrane (Micron Separations Inc., Westboro, Ma, USA) and degassed before use. Vitamins were supplied by Sigma Chemical Company. All other chemicals were of analytical reagent grade and purchased from Mallinckrodt Chemical (St. Louis, USA) and Merck (Darmstadt, Germany).

2.2 Biodegradability of Disinfectants in Surface Waters

Surface water samples were taken from 18 sampling points covering the urban area of Buenos Aires. They were refrigerated at 4 °C until processed within 6 h. Samples were supplemented with 20 mg L^{-1} of each disinfectant and incubated for 10 days at 20 °C in a respirometric apparatus (BOD track II®-Hatch). Each sample was performed in duplicate. Control consisted of surface water without any disinfectant and was simultaneously conducted. The final volume in the apparatus was 350 mL. Samples were magnetically stirred throughout incubation; oxygen consumption was continuously measured. Samples with oxygen consumption values significantly greater than control were selected for testing the presence of degrading bacteria. A disinfectant is assumed to be inhibitory if less than 25% oxygen consumption has occurred in comparison with control (OECD 1992).

Water samples were characterized according to Standard Methods for the Examination of Water and Wastewater (APHA 2012). The following determinations were carried out: chemical oxygen demand (COD), biochemical oxygen demand (BOD), total heterotrophic bacteria, *Escherichia coli*, and Enterococcus counts.

2.3 Selection and Identification of Degrading Bacteria

The use of a disinfectant as sole carbon source was tested inoculating 100 mL of minimal mineral medium supplemented with the disinfectant as sole carbon source (20 mg L^{-1}) with 1 mL of the sample selected for the biodegradability assay. Samples were incubated at 28 °C in a rotatory shaker (200 rpm) for 7 days. Disinfectant concentration was measured daily. Bacterial communities able to degrade the compound in minimum time were selected for the subsequent biodegradation assays.

The synthetic minimal medium was previously described (Korol et al. 1989). It contained (g L^{-1}) 1.73 g K_2HPO_4; 0.68 g KH_2PO_4; 0.83 g $(NH4)_2SO_4$, and 0.1 g $MgSO_4.7H_2O$ (final pH 7.4) The medium was supplemented by a trace elements solution and a stock vitamin solution. Both solutions were sterilized by filtration and added aseptically to the autoclaved minimal medium. The trace elements solution was added at a concentration of 0.5% v/v^{-1}, whereas, vitamin solution was added at a concentration of 0.1% v/v^{-1}. The composition of the trace elements solution was (g L^{-1}) 1 g $CaCl_2.2H_2O$; 0.3 g $MnSO_4.H_2O$; 0.5 g $FeSO_4.7H_2O$ and 0.2 g hydrated disodium salt of EDTA. The stock vitamin solution contained 40 mg of calcium pantothenate, 2 mg of folic acid, 200 mg of inositol, 40 mg of nicotinic acid, 20 mg of p-aminobenzoic acid, 40 mg of pyridoxine hydrochloride, 20 mg of riboflavin, and 40 mg of

thiamine hydrochloride in 100 mL of distilled water. Bacteria isolation was carried out onto a tryptone soy agar medium (Merck, Darmstadt, Germany) supplemented with 20 mg L^{-1} of the disinfectant.

Degrading bacteria were further characterized by Gram staining and identified both by conventional biochemical test and the API20NE ® system (BioMerieux, L'Etoile, France). For further identification, 16S rRNA gene sequencing was employed. The 16S ribosomal RNA (rRNA) gene was amplified by polymerase chain reaction (PCR) using the following primers (50e30) 16SR: GYTACCTTGTTACGACTT, 16SF: AGAGTTTG ATCMTGGCTCAG and heat extracted DNA as template. Amplified fragments were purified with the QIAquick PCR purification kit (QIAGEN) and sequenced in both strands using an ABI Prism DNA 3700 sequencer. Nucleotide sequences were compared with databases using the NCBIs' Basic Local Alignment Search Tool (Blast).

2.4 Biodegradation Assay in Batch Reactor

The bacterial strain was adapted by inoculation in the synthetic minimal medium previously described supplemented with 100 mg L^{-1} BAC and incubated in a rotary shaker at 28 °C for 48 h to provide stock culture.

Biodegradation assays were performed in a New Brunswick Multigen TA microfermentor, aerobically operated at 28 °C with an effective volume of 1250 mL of minimal medium supplemented with the corresponding amount of BAC as the sole carbon source to obtain the desired value of initial concentration according to the specific assay (100, 200, and 500 mg L^{-1}). The system was inoculated with 5 mL of the stock culture (final concentration 10^6 cells mL^{-1}). During incubation, 10 mL samples were removed from the system at appropriate intervals in order to determine the amount of remaining BAC and to evaluate microbial growth. Abiotic loss of the compound was estimated in a control assay without inoculation.

2.5 Analytical Methods and Control Parameters

To determine the amount of remaining BAC, bacterial cells were separated by centrifugation. The filtered supernatant fluid was analyzed spectrophotometrically (Metrolab UV 1700 Spectrophotometer) using a modification of the direct colorimetric method proposed by Lowry (1979) (Baroni et al. 2016). To assess, mineralization selected samples were analyzed by high performance chromatography (HPLC).

Determination of cell viability in batch reactors was performed by spreading sample dilutions on the surface of tryptone soy agar plates. COD was measured in samples taken at the beginning and at the end of the batch process according to APHA (2012).

2.6 Instrumentation and Chromatographic Conditions

The Spectra System HPLC equipment assembled comprised a Thermo Scientific SCM1000 Quaternary pump, a P4000 degasser, an AS3000 autosampler, and a UV2000 Dual λ Absorbance detector. Analysis of chromatograms was carried out using a ChromQuest Chromatography Data System software. Separation of BAC was achieved with a Symmetry RP-18 (75 mm × 4.6 mm i.d, 3.5 μm particle size). The chromatographic condition was as follows: an injection volume of 25 μL, a column temperature of 50ΊC with an isocratic mobile phase prepared with a mixture of methanol: potassium dihydrogen phosphate (7.5 mM, pH 3.0; 70:30, v/v^{-1}) and a flow rate of 1.0 mL min^{-1}. UV-detection was performed at λ 208 nm. Separation of BAC was accomplished in less than 6 min (Labranche et al. 2007).

2.7 Toxicity Tests

Detoxification was evaluated by *Vibrio fischeri*, *Pseudokirchneriella subcapitata*, *and Lactuca sativa* toxicity tests. Toxicity tests were performed in samples taken at the beginning and at the end of the biodegradation process.

2.7.1 Luminescent Bacteria Test

The Microtox acute toxicity test uses *Vibrio fischeri*, a marine luminescent bacterium (strain NRRL B-11177) as test organism. The bacterial strain was purchased from Strategic Diagnostic Inc. (Carlsbad, CA, USA) as a freeze-dried form. The Microtox Model 500 Toxicity Analyzer (Azur Environmental, Carlsbad, CA, USA) was used to do the Microtox test. Toxicity was assessed by measuring the inhibition of light emission of *Vibrio fischeri*. The bacteria were exposed to a series of sample dilutions for 15 min at 15 ± 1 °C, according to ISO 11348–3 (1998). Results are expressed in effective concentration 50 (EC_{50}), concentration which provokes a 50% reduction in bacterial light emission relative to the control test after a

period of exposure of 15 min. EC_{50} was determined using the MicrotoxOmni software. Before assays were performed, the organism sensitivity was evaluated using phenol as the reference toxic compound.

2.7.2 Alga Test

Toxicity was assessed by measuring growth inhibition of the alga *Pseudokirchneriella subcapitata*, exposed for 72 h, according to ISO 8692 (2004). *Pseudokirchneriella subcapitata* growth inhibition tests were performed at 23 ± 2 °C, under continuous white light conditions. Results are expressed in effective concentration 50 (EC_{50}), concentration which provokes a 50% reduction in alga growth relative to control after a period of exposure of 72 h. Algal cell concentration was measured by direct enumeration and EC_{50} was estimated by the graphical method. Before assays were performed, the organism sensitivity was evaluated; potassium dichromate was used as the reference toxic compound.

2.7.3 Seed Test

Toxicity was assessed by measuring reduction in root elongation of *Lactuca sativa* according to EPA/600/3-88 (1989). Lettuce seeds were exposed to different sample concentrations. Ten lettuce seeds were placed in Petri dishes with one dish for each concentration on wet filter papers for 120 h at 24 ± 2 °C in the dark. Results are expressed in effective concentration 50 (EC_{50}), concentration which provokes a 50% inhibition of lettuce root elongation compared to control after the 120-h period of exposure. Before assays were performed, organism sensitivity was evaluated using zinc sulfate as the reference toxic compound.

3 Results

3.1 Biodegradability of Disinfectants in Surface Waters

A total of 18 sampling points were selected, six of them were located in the Reconquista basin, six in the Matanza-Riachuelo basin, and six in the La Plata River. In Fig. 1, a schematic map of the study area is shown. Characterization of surface water samples is shown in Table 1.

In biodegradability assays, BCA showed oxygen consumption values above the control in 14 sampling points (Fig. 2); in all cases, the use of BCA as the only

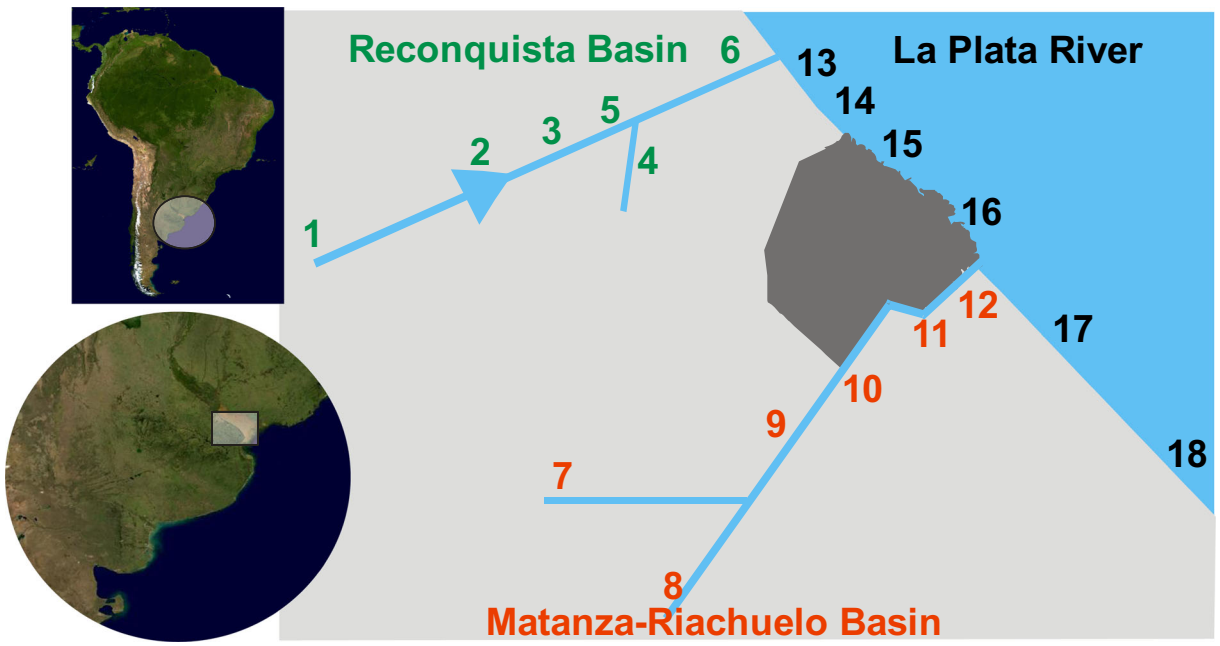

Fig. 1 Location of sampling points. *Reconquista Basin*: 1, Durazno stream; 2, Roggero dam; 3, Merlo; 4, Route 8 bridge; 5, Morón stream; 6, Tigre. *Matanza-Riachuelo Basin*: 7, Morales stream; 8, Rodríguez stream; 9 Ricchieri highway bridge; 10 La Noria bridge; 11, Alsina bridge; 12, La Boca. *La Plata River*: 13, San Isidro Port; 14 Olivos Port; 15, Costanera Norte; 16, Costanera Sur; 17, Quilmes; 18, Punta Lara

Table 1 Characterization of surface waters samples

No.*	Sampling point	BOD_5 ($mg\ O_2\ L^{-1}$)	COD ($mg\ O_2\ L^{-1}$)	Total heterotrophic bacteria ($CFU\ mL^{-1}$)	Thermotolerant coliforms ($CFU\ mL^{-1}$) ($MPN\ 100\ mL^{-1}$)**	*Escherichia coli* ($CFU\ mL^{-1}$) ($MPN\ 100\ mL^{-1}$)**	Enterococcus ($CFU\ mL^{-1}$) ($MPN\ 100\ mL^{-1}$)**
1	Durazno stream	< 5	38	4.0×10^3	*460*	*460*	*93*
2	Roggero dam	13	88	1.9×10^3	*7.3*	*7.3*	*43*
3	Merlo	20	125	1.2×10^7	5.7×10^3	2.0×10^3	8.5×10^2
4	Route 8 bridge	23	76	1.0×10^6	4.7×10^3	3.2×10^3	9.2×10^2
5	Morón stream	53	127	8.0×10^5	2.8×10^3	1.7×10^3	9.0×10^1
6	Tigre	< 5	43	2.4×10^4	1.3×10^3	1.5×10^1	*93*
7	Morales stream	6	69	1.6×10^5	3.1×10^3	3.0×10^2	5.0×10^1
8	Rodríguez stream	< 5	29	1.6×10^4	8.0×10^1	*3.0×10^1*	2.0×10^1
9	Ricchieri highway bridge	9	89	5.0×10^4	1.2×10^3	8.0×10^1	2.0×10^1
10	La Noria bridge	5	90	5.6×10^5	3.3×10^3	1.3×10^3	3.7×10^2
11	Alsina bridge	26	79	1.7×10^5	1.0×10^3	4.2×10^2	4.0×10^1
12	La Boca	28	82	3.5×10^5	7.5×10^3	2.5×10^3	2.5×10^2
13	San Isidro Port	< 5	< 3	3.8×10^3	3.5×10^1	1.0×10^1	*36*
14	Olivos Port	< 5	7	7.2×10^4	4.5×10^2	2.0×10^2	*1100*
15	Costanera Norte	< 5	42	1.4×10^4	2.2×10^2	5.0×10^1	*75*
16	Costanera Sur	< 5	49	8.0×10^4	6.5×10^2	2.2×10^2	*64**
17	Quilmes	< 5	5	6.0×10^3	5.0×10^1	*1100*	*240*
18	Punta Lara	< 5	32	1.2×10^4	3.0×10^1	*5.0×10^1*	*43*

* No. number assigned for the sampling point

** MPN counts in italics

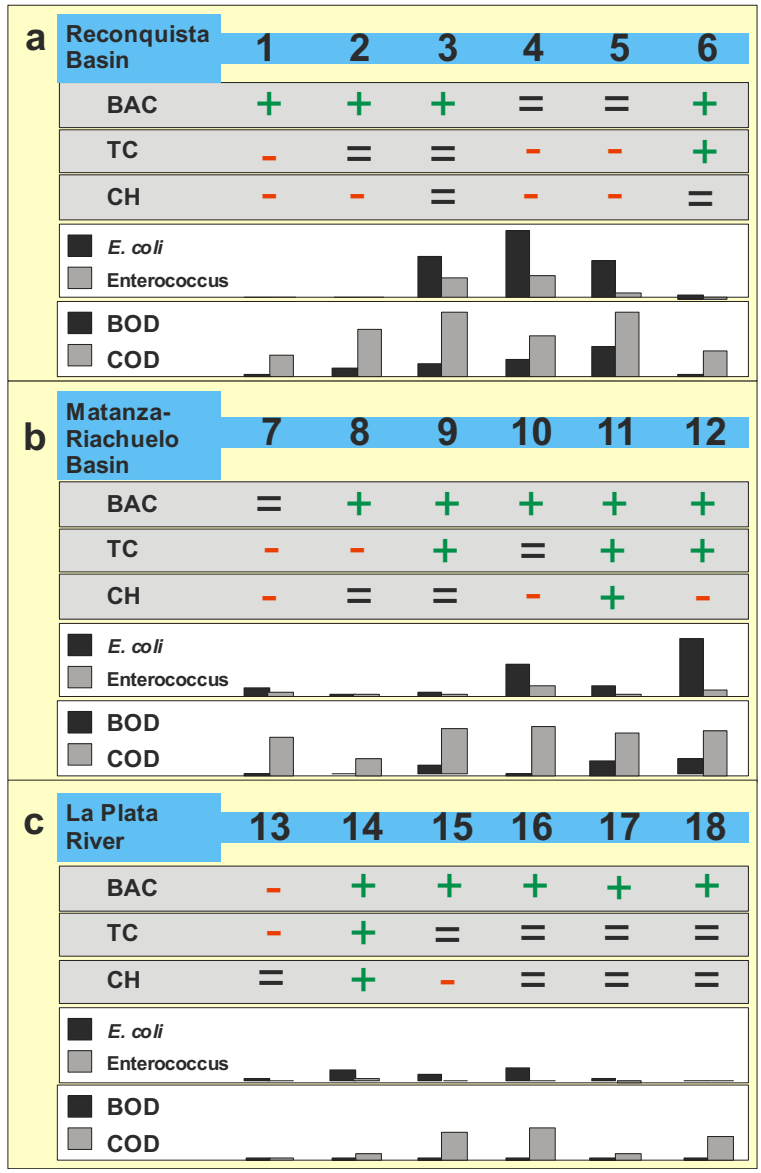

Fig. 2 Biodegradability assay of BAC, TC and CH in surface waters from the Reconquista basin (**a**), the Matanza-Riachuelo basin (**b**) and the La Plata River (**c**). Oxygen consumption after 10 days above control (+); oxygen consumption after 10 days similar to control (=); oxygen consumption after10 days below control (−)

source of carbon was later confirmed. Batch assays with the selected BCA degrading communities, proved that bacteria isolated from the Punta Lara sample have the highest degradative capacity and was selected to perform subsequent assays (data not shown).

The other two disinfectants proved to be markedly less biodegradable. In the case of TC, samples showed higher oxygen consumption than control in only five sampling points (Fig. 2). The use of TC as the sole carbon source was proved in the La Boca sample only. In the other cases, a partial degradation may have taken place. (The selected community was employed in later studies that are not shown in this work). Finally, only two of the samples supplemented with CH showed higher oxygen consumption than control and, in this case, it was not possible to prove the use of the compound as the only source of carbon (Fig. 2).

When the compound is not degraded, there are two possibilities: the water bacterial communities may not have been affected, or they may have been inhibited, inhibition would be shown by an oxygen consumption lower than control. This inhibitory effect was most markedly present for CH (8 sampling

points), followed by TC (6 sampling points), and lastly, BAC (1 sampling point). On the contrary, it should be noted that biodegradation and tolerance were the predominant effects for all the disinfectants tested, with an occurrence of 94% of the sampling points for BAC, 67% for TC, and 56% for CH.

3.2 Selection and Identification of BCA Degrading Bacteria

The isolated bacterial strain from the Punta Lara sampling point was a Gram negative bacillus. This strain did not ferment glucose, was oxidase negative, unable to reduce nitrate to nitrite, and mobile. Using API 20 NE, the strain was identified as belonging to the genus *Pseudomonas* with 99.6% probability. The strain was able to use glucose, arabinose, maltose, mannitol, N-acetylglucosamine, gluconate, capric acid, malic acid, citrate, and phenylacetic acid as carbon sources. The test carried out showed that neither maltose nor adipic acid could be used as carbon sources. Partial 16S rRNA gene sequencing confirmed 99% homology with the genus *Pseudomonas*. (E value = 0, p value < 0.05).

3.3 Biodegradation Assay in Batch Reactor

Biodegradation assays were carried out with initial inocula of 1×10^6 cells mL^{-1}. The assays showed that the indigenous *Pseudomonas* strain was capable of degrading 100 mg L^{-1} of BCA in 10 h (Fig. 3a). Specific growth rate (μ) was 0.56 h^{-1} (95% confidence interval–CI: 0.45–0.68). Removal of the compound was 99.5% of initial concentration, with a 93.8% removal of COD. The control assay showed the absence of abiotic loss in the system (data not shown). Complete degradation of BCA was proved by HPLC performed at the end of the batch process (Fig. 4). When the initial concentration of BAC was raised to 200 mg L^{-1}, the process took 25 h (Fig. 3b) and the specific growth rate decreased to 0.30 h^{-1} (95% CI: 0.27–0.33). Removal efficiency was of 99.0 and 90.3% respectively, expressed in terms of compound and COD. The higher initial concentration that could be completely degraded was of 500 mg L^{-1}. In this case, specific growth rate (μ) was even lower (Fig. 3c). A value of 0.14 h^{-1} (95% CI: 0.12–0.16) was obtained and degradation was accomplished in 46 h with an efficiency of 98.0% in terms of compound removal and 89.1% in terms of COD removal.

3.4 Toxicity Tests

Bioassays of acute toxicity were conducted in order to confirm total elimination of toxicity from the synthetic effluent as a result of the biodegradation process. Table 2 shows that toxicity levels at the beginning of the assay were high for all the organisms tested, whereas no toxicity was detected after the biodegradation process.

3.5 Discussion

The selected sampling points as a whole cover a representative area of waters in the urban Buenos Aires region. Both the Matanza-Riachuelo and the Reconquista basins are two of the most polluted basins in Argentina (Salibián 2006; ACUMAR 2015). By comparison, the La Plata River is markedly less polluted, to the extent that it is used as the source of drinking water for the City of Buenos Aires. It forms a great estuary into which two of the most important rivers of South America, Paraná, and Uruguay rivers, flow. However, along the urban coastal area of Buenos Aires, it receives contaminated water from numerous waterways including those of the above-mentioned Reconquista and Matanza-Riachuelo basins (Natale 2005).

Unfortunately, it was not possible to determine disinfectants concentration in water courses, but as it is generally considered that the main source of these contaminants is sewage water, (Jones et al. 2001; Kümpel et al. 2001) studies were carried out in order to correlate the presence of degrading bacteria and fecal pollution. It was expected that bacterial pre-exposition to the compounds would facilitate the selection of biodegradative strains (Alexander 1999). However, the presence of disinfectant degrading bacterial communities did not correlate with fecal pollution. For example, BAC degrading bacteria were isolated both from relatively unpolluted rural areas, such as the Durazno stream in the upper Reconquista basin, and from sampling points, for instance Puente Alsina on the Riachuelo River, a paradigm of pollution in the region. It should be noticed that the presence of degrading bacteria in the samples without oxygen consumption above control cannot be excluded, since the assay time is relatively short and the assay is conducted without a prior compound adaptation step.

The degree of biodegradability obtained for the three compounds is that predicted and reported in the literature (EPA 2017, Sütterlin et al. 2008a, Federle et al. 2002, Das et al. 2015). BCA proved to be the most easily

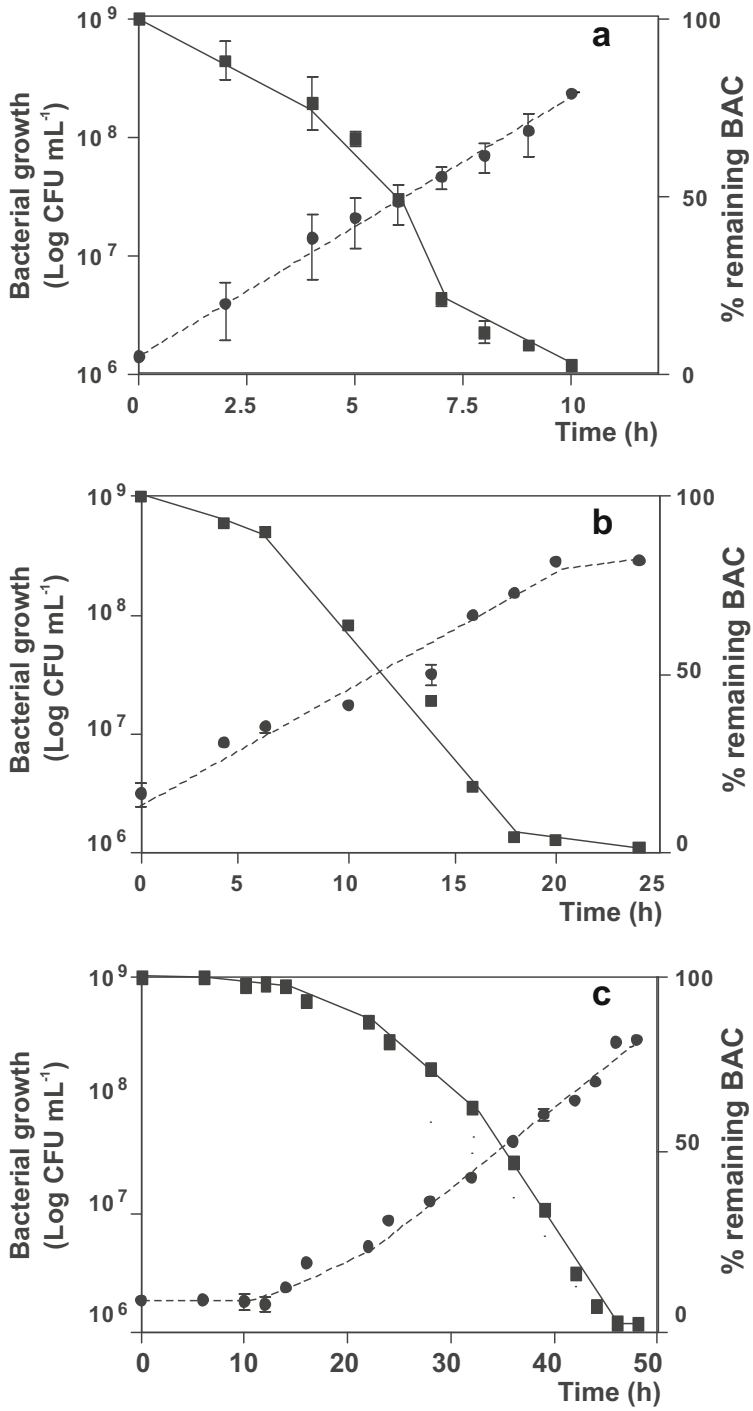

Fig. 3 Degradation of BAC by *Pseudomonas* sp. in batch reactor. **a** Initial concentration of BAC 100 mg L^{-1}; **b** initial concentration of BAC 200 mg L^{-1}; **c** initial concentration of BAC 500 mg L^{-1}

biodegradable compound and CH the most persistent. Beyond biodegradability, perhaps more relevant data may be obtained from the analysis of the impact of the presence of disinfectants on bacterial communities in water. In 94% of the sampling points, 20 mg L^{-1} of BAC did not affect oxygen consumption. It should be considered that QACs are known to be effective at low concentrations (<1 mg L^{-1}) against bacteria (Kümmerer et al. 2004b). Likewise, percentages of 67% for TC and 56% for CH were obtained. Reported concentrations for the inhibition

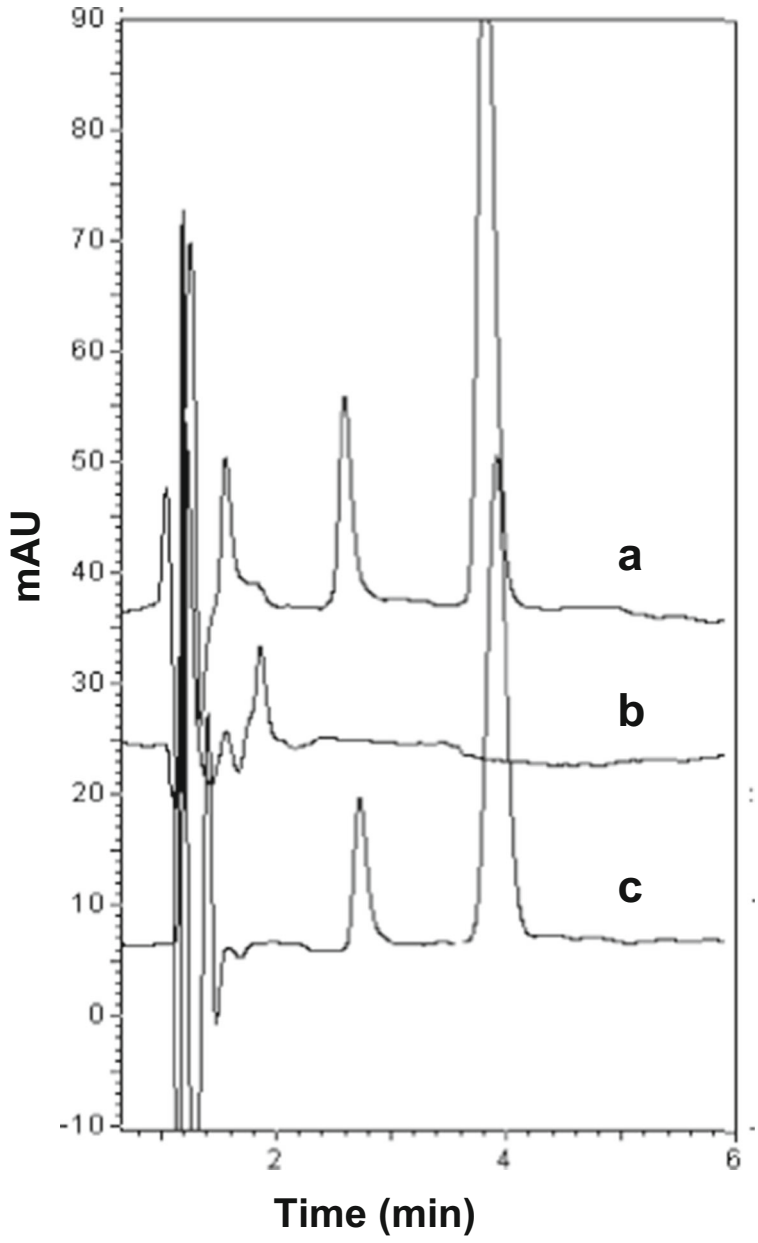

Fig. 4 HPLC chromatograms: (a) initial batch process; (b) final batch process; (c) BAC standard

Table 2 Biodegradation of BAC in batch reactors

Test organisms	EC_{50} initial % *v/v*	EC_{50} Final % *v/v*
Vibrio fischeri	0.41	> 90
Pseudokirchneriella subcapitata	1.03	> 90
Lactuca sativa	0.36	> 100

Toxicity tests

of activated sludge communities were of 1.8 mg L^{-1} for TC (Neumegen et al. 2005) and much lower for CH (Freitag et al. 1982). This result means that water populations are already resistant to the disinfectant, and that this occurs without a correlation with fecal pollution, the most likely source of exposure to the compounds. This tolerance can surprisingly be seen even in the La Plata River, where bacterial communities also show high tolerance to all the disinfectants tested. One possible explanation could be

that the whole study area is given over to intense agro-industrial activities. It is known that in a large number of these activities, such as in industrial livestock production, biocidal products are employed. Taking into account that there may be cross-resistance between antibiotics and disinfectants (Koäljalg et al. 2002; Tandukar et al. 2013), the reason for such high tolerance could be previous exposure of the indigenous communities to any of the agents released. Further studies are needed to confirm this hypothesis.

Despite being relatively biodegradable, BAC is an inhibitory compound for bacterial populations, biodegradation in water treatments plants taking place at very low rates (Zhang et al. 2011). Moreover, due to their potential environmental impact, products containing BAC or other QACs have been eliminated by Freiburg University Hospital for a number of years now (Kümmerer 2001b). As a consequence of their low biodegradability, combinations of physicochemical and biological methods have been proposed for their degradation (Loveira et al. 2012). Without ignoring the versatility of physicochemical processes, they can hardly compete in terms of cost with biological processes when a single chemical species is involved (Fortunato et al. 2016). Therefore, the selection of indigenous species capable of metabolizing high concentrations of problematic compounds is an attractive strategy either for bioaugmentation processes or for the treatment of partial effluents containing the compound.

The isolated strain from the Punta Lara sampling point, which is the strain aimed principally at in this study, was identified as belonging to the genus *Pseudomonas*. This genus is known for the diversity of carbon sources it uses, there are also reports in the literature of indigenous *Pseudomonas* strains capable of degrading persistent compounds, e.g., BAC (Loveira et al. 2012; Khan et al. 2015). The isolated *Pseudomonas* strain was able to metabolize up to 500 mg L^{-1} of BAC in times compatible with a biological treatment. These concentration values are significantly higher than those reported in other studies. Loveira et al. (2012) informed of the toxic effect on bacterial biofilm in biological reactors at a concentration of 180 ml L^{-1}. Khan et al. (2015) evaluated the growth of a strain of *Pseudomonas* sp. using benzyl dimethyl dodecyl ammonium chloride (BDDA), one of the homologs constituting the commercial BKC, as the sole carbon source. The degradation of a concentration of 100 mg L^{-1} of the compound took 150 h. Inhibitory effects began when the concentration increased to 150 mg L^{-1}. Nishihara et al. (2000) isolated a strain of *Pseudomonas fluorescens* able to degrade 50 mg of another BAC homolog, didecyldimethylammonium chloride, within 7 days. Tezel and Pavlostathis (2009) investigated the transformation of BCA into batch assays under nitrate reduction conditions using mesophilic methanogenic bacteria as inoculum. The concentration was reduced from 110 mg L^{-1} to 10.8 mg L^{-1} over a period of 42 days. Bacteria from genera *Aeromonas* (Patrauchan and Oriel 2003) and *Acinetobacter* (Al-Ahmad et al. 2000) were also involved in BAC degradation.

When initial BAC concentration increases from 100 to 500 mg L^{-1}, bacterial growth rate decreases. This is characteristic of toxic substrates and further evidence of BAC inhibitory action (Alexander 1999).

HPLC results associated with the decrease in COD values and the absence of UV absorbance demonstrated the mineralization of the compound and the absence of metabolites. Despite the high values of initial toxicity, similar to those reported by other authors (Sütterlin et al. 2008b), a complete detoxification was obtained as a result of the treatment.

4 Conclusion

In conclusion, this study found that samples from 14 of the 18 sampling points tested were able to degrade BAC and one was able to degrade TC. Moreover, a high tolerance to all disinfectants in the area was found. The concentration of 20 mg L^{-1} of BAC employed did not inhibit bacterial communities in 94% of the sampling points. At the same concentration, this value was 67% for TC and 56% for CH. Neither the tolerance nor the biodegradation correlated with fecal contamination in the waterways. The indigenous strain isolated from Punta Lara showed the highest degradation capability and was identified as belonging to the genus *Pseudomonas*. Degradation efficiency for an initial BAC concentration of 100 mg L^{-1} was 99.5% expressed as compound removal and 93.8% in terms of COD removal. Using *Pseudokirchneriella subcapitata*, *Vibrio fischeri*, and *Lactuca sativa* as test organisms, complete detoxification was assessed at the end of the process.

The ability of the isolated indigenous strain could be exploited to improve the treatment of wastewaters containing high concentrations of BAC, either for bioaugmentation processes or partial effluents treatment containing the

compound. As well as studying the widespread pollution caused by the use of disinfectants, usually involving very low concentrations of the products, point contamination caused by effluents from the pharmaceutical and cosmetics industries should also be considered.

Acknowledgements We also thank Dr. Gabriel Gutkind and Dr. Marcela Radice from the Laboratory of Bacterial Resistance, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, for his collaboration in the identification of microorganisms.

Funding Information We thank the University of Buenos Aires for the grant given for this study, supported by UBACYT Program-Project 20020130100378BA 2014-2017.

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