



Technology

ISSN: 0959-3330 (Print) 1479-487X (Online) Journal homepage: http://www.tandfonline.com/loi/tent20

Aerobic degradation of ibuprofen in batch and continuous reactors by an indigenous bacterial community

María Susana Fortunato, Nancy Piedad Fuentes Abril, Manuela Martinefski, Valeria Trípodi, Mariana Papalia, Marcela Rádice, Gabriel Gutkind, Alfredo Gallego & Sonia Edith Korol

To cite this article: María Susana Fortunato, Nancy Piedad Fuentes Abril, Manuela Martinefski, Valeria Trípodi, Mariana Papalia, Marcela Rádice, Gabriel Gutkind, Alfredo Gallego & Sonia Edith Korol (2016): Aerobic degradation of ibuprofen in batch and continuous reactors by an indigenous bacterial community, Environmental Technology, DOI: <u>10.1080/09593330.2016.1156773</u>

To link to this article: http://dx.doi.org/10.1080/09593330.2016.1156773



Accepted author version posted online: 23 Feb 2016. Published online: 28 Mar 2016.



 ${\mathscr S}$ Submit your article to this journal ${\mathbb C}$

Article views: 39



View related articles 🗹



View Crossmark data 🕑

Full Terms & Conditions of access and use can be found at http://www.tandfonline.com/action/journalInformation?journalCode=tent20

Aerobic degradation of ibuprofen in batch and continuous reactors by an indigenous bacterial community

María Susana Fortunato^a, Nancy Piedad Fuentes Abril^a, Manuela Martinefski^b, Valeria Trípodi^c, Mariana Papalia^d, Marcela Rádice^d, Gabriel Gutkind^d, Alfredo Gallego^a and Sonia Edith Korol^a

^aCátedra de Salud Pública e Higiene Ambiental, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina; ^bCátedra de Química Analítica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina; ^cCátedra de Calidad de Medicamentos, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina; ^dLaboratorio de Resistencia Bacteriana, Cátedra de Microbiología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina;

ABSTRACT

Water from six points from the Riachuelo-Matanza basin was analyzed in order to assess ibuprofen biodegradability. In four of them biodegradation of ibuprofen was proved and degrading bacterial communities were isolated. Biodegradation in each point could not be correlated with sewage pollution. The indigenous bacterial community isolated from the point localized in the La Noria Bridge showed the highest degradative capacity and was selected to perform batch and continuous degradation assays. The partial 16S rRNA gene sequence showed that the community consisted of *Comamonas aquatica* and *Bacillus* sp. In batch assays the community was capable of degrading 100 mg L⁻¹ of ibuprofen in 33 h, with a specific growth rate (μ) of 0.21 h⁻¹. The removal of the compound, as determined by High performance liquid chromatography (HPLC), exceeded 99% of the initial concentration, with a 92.3% removal of Chemical Oxygen Demand (COD). In a down-flow fixed-bed continuous reactor, the community shows a removal efficiency of 95.9% of ibuprofen and 92.3% of COD for an average inlet concentration of 110.4 mg. The reactor was kept in operation for 70 days. The maximal removal rate for the compound was 17.4 g m⁻³ d⁻¹. Scanning electron microscopy was employed to observe biofilm development in the reactor. The ability of the isolated indigenous community can be exploited to improve the treatment of wastewaters containing ibuprofen.

Introduction

In recent years a new type of pollutants has attracted the attention of research groups, particularly in developed countries. These compounds are found in low concentrations and most remain unregulated.[1,2] These contaminants are called emerging contaminants or microcontaminants [3] and comprise a heterogeneous group composed mainly of pharmaceutical products, personal care products, surfactants, industrial additives, plasticizers and pesticides, among a wide variety of chemical compounds. In the case of pharmaceuticals, these compounds are developed to have high biological activity, which along with its high consumption and high persistence transform them into a potential problem in the environment.[4]

Available information about these compounds in different countries shows that the presence of drugs in the environment is a widespread problem.[5] This is demonstrated by research conducted in Finland, [6] France, Greece, Italy, [7] Spain, [8,9] Slovenia, [10]

Sweden [11] and Switzerland. [12,13] It is worth noting that the drug concentrations found in environmental samples are characteristic of each region and are related to the volumes sold by the market and the prescription pattern.[14] The main pharmaceutical products studied in terms of their occurrence in the environment can be divided into the following treatment groups: nonsteroidal anti-inflammatory drugs (NSAIDs: ibuprofen, diclofenac, paracetamol and aspirin), antiepileptic drugs (carbamazepine), stimulants (caffeine), ß-blockers (atenolol and propranolol), antibacterials (erythromycin, tetracycline, oxytetracycline, sulfamethoxazole, trimethoprim, and chloramphenicol), hormones (oral contraceptives, testosterone, estrogen, estradiol, estrone, and 17a-ethinylestradiol), and antineoplastic (ifosfamide and cyclophosphamide) and lipid-lowering agents (clofibric acid and bezafibrate).[6,14-17]

Unlike other chemicals of industrial origin, most pharmaceutical products reach aquatic ecosystems

ARTICLE HISTORY

Received 27 December 2015 Accepted 16 February 2016

Taylor & Francis

Taylor & Francis Group

KEYWORDS

Emerging contaminants; ibuprofen; aerobic reactors; biodegradation; Riachuelo-Matanza basin

CONTACT Alfredo Gallego agallego@ffyb.uba.ar Cátedra de Higiene y Sanidad, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, Buenos Aires 1113, Argentina

 $[\]ensuremath{\mathbb{C}}$ 2016 Informa UK Limited, trading as Taylor & Francis Group

permanently, due to widespread use in human and veterinary medicine. The drugs after complying their effect on the human or animal body are mainly eliminated through urine and feces either unchanged, conjugated with any molecule in order to make them more hydrophilic (glucuronides or sulfates), or as a mixture of metabolites. [15,18,19] Also the discharge of sewage, industrial or agricultural-livestock effluents is a major source of contamination of waterways with these compounds.[20-23] Sewage treatment plants are not usually designed to carry out an efficient degradation of such persistent compounds. Thus, a high proportion of pharmaceuticals and their metabolites undergo no alteration to go through them and can reach the aquatic environment, both surface water and groundwater.[2,24] For example, the anti-inflammatory ibuprofen used in large quantities for the treatment of pain, dysmenorrhea and inflammation is metabolized in sewage treatment plants with varying removal rates depending not only on the applied method but also on other variables within a particular technology applied, such as effluent dilution or reactor configuration.[12] Studies in Germany showed the presence of not only ibuprofen but also its metabolite hydroxy ibuprofen in water from rivers Elbe and Saale. [25] One of the facts that has attracted the most concern and demonstrates the reach of the problem is the detection of some of these drugs, such as ibuprofen, diclofenac, carbamazepine or clofibric acid, in drinking water.[26,27]

Although drugs are designed to maintain a specific action in both humans and animals, they also have undesirable side effects. Once these products enter the environment, they can affect living beings causing a wide range of effects not necessarily related to its therapeutic action.[28] For example, some NSAIDs such as ibuprofen, naproxen, acetylsalicylic acid and diclofenac, widely used worldwide, exert an effect on the mobility and growth of certain species of algae, invertebrates and fish.[29]

Biodegradation is the most important process in treatment plants for eliminating potential contaminants from wastewater.[30] The selection of indigenous bacterial strains capable of degrading these compounds and the use of bioaugmentation strategies may be an appropriate strategy to achieve greater process efficiency. Also, the reactor design should be adapted to the treatment requirements. Biofilm reactors have proven particularly suitable for the treatment of toxic compounds, because bacteria in the biofilm are more resistant than that in suspension. Biofilms can often be formed by communities of microorganisms.[31]

In Argentina, the National Drug, Food and Medical Technology Administration (ANMAT) does not consider the environmental risk assessment in relation to the introduction of new drugs on the market. Neither are there studies on biodegradability of pharmaceuticals in surface waters in our region.

The purpose of the present investigation was (a) to study the biodegradability of ibuprofen in surface waters from the Riachuelo-Matanza basin in laboratory scale and try to correlate it with fecal contamination of water, (b) to isolate the microorganisms involved in the biodegradation process and (c) to apply selected microorganisms in batch and continuous treatment of synthetic wastewater contaminated with ibuprofen.

Material and methods

Biodegradability of ibuprofen in surface waters

Surface water samples from the Matanza-Riachuelo basin were added with 20 mg L^{-1} of ibuprofen and incubated for 10 days at 20°C in a Respirometric Apparatus (BOD track II[°]-Hatch). The final volume in the apparatus was 350 mL and it was magnetically stirred throughout the whole incubation. Each sample was performed in duplicate. A control consisting of surface water without compound was jointly conducted. Oxygen consumption was continuously measured. Samples with oxygen consumption values greater than that of the control were selected for testing the presence of ibuprofen-degrading bacteria.

In order to characterize water samples, the following determinations, according to Standard Methods for the Examination of Water and Wastewater,[32] were carried out: chemical oxygen demand (COD), biochemical oxygen demand (BOD), total heterotrophic bacteria, *Escherichia coli*, and Enterococcus count.

Selection and identification of ibuprofendegrading bacteria

The use of ibuprofen as the sole carbon source was tested as follows. Minimal mineral medium supplemented with ibuprofen (20 mg L^{-1}) was inoculated with 1% of the sample selected from the previous assay.

The composition of the synthetic minimal medium was (g L⁻¹) as follows: 1.73 g K₂HPO₄; 0.68 g KH₂PO₄; 0.83 g (NH4)₂SO₄ and 0.1 g MgSO₄·7H₂O (final pH 7.4). A trace elements solution and a stock vitamin solution were sterilized by filtration and added aseptically to the autoclaved synthetic minimal medium. The trace elements solution was added at a concentration of 0.5% v v⁻¹. It contained (g L⁻¹): 1 g CaCl₂·2H₂O; 0.3 g MnSO₄·H₂O; 0.5 g FeSO₄·7H₂O and 0.2 g hydrated disodium salt of Ethylenediaminetetraacetic acid. The composition of the stock vitamin solution was 40 mg calcium pantothenate, 2 mg folic acid, 200 mg inositol, 40 mg nicotinic acid, 20 mg *p*-aminobenzoic acid, 40

mg pyridoxine hydrochloride, 20 mg riboflavin and 40 mg thiamine hydrochloride in 100 mL of distilled water. The vitamin solution was added at a concentration of $0.1\% \text{ v v}^{-1}$. For the communities able to degrade ibuprofen, we intended the isolation of the individual bacteria in tryptone soy agar (TSA) (Merck, Darmstadt, Germany) containing 20 mg L^{-1} of ibuprofen, in order to maintain the selective pressure. Individual strains were preserved in TSA slants. To prove the use of ibuprofen as the sole carbon source, the following assay was carried out with each individual strain. A 24 h culture in a TSA slant containing 20 mg L^{-1} of ibuprofen for the strain was used to obtain a bacterial suspension, equivalent to the second tube of the McFarland scale. One milliliter of the suspension was used to inoculate Erlenmeyer flasks containing 100 mL of minimal medium with ibuprofen (50 mg L^{-1}) as the sole carbon source. The flasks were incubated with rotatory shaking at 28°C for seven days. Ibuprofen degradation and bacterial growth were measured as described in the biodegradation assays.

Bacteria capable of degrading ibuprofen was further characterized by Gram staining and identified both by conventional biochemical test and using 16S rRNA gene sequencing. 16S ribosomal RNA (rRNA) gene was amplified by the polymerase chain reaction (PCR) using the following primers (50e30) 16SR: GYTACCTTGTTAC-GACTT, 16SF: AGAGTTTGATCMTGGCTCAG, and heatextracted 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).

Chemicals

Ibuprofen used for the production of medicinal preparations (purity 99%) was obtained from a local pharmaceutical company. An ibuprofen stock solution (2000 mg L^{-1}) was prepared by dissolving the necessary amount in 0.1 N NaOH and sterilized by filtration. Phosphoric acid (85%) analytical grade was supplied by Mallinckrodt (New York, U.S.A.) and methanol was supplied by Sintorgan (Buenos Aires, Argentina). Ultrapure water was obtained by an EASY pure RF equipment (Barnstead, Dudubuque, IA, U.S.A.). All solutions used for HPLC were filtered through a 0.45 µm nylon membrane (Micron Separations Inc., Westboro, MA, U.S.A.) and degassed before use. Vitamins were supplied from Sigma Chemical Company. All the other chemicals were of analytical reagent grade and purchased from Mallinckrodt Chemical (St. Louis, U.S.A.) and Merck (Darmstadt, Germany).

Biodegradation assay in batch reactor

Bacterial community was adapted by inoculation in the synthetic minimal medium previously described supplemented with 100 mg L^{-1} of ibuprofen as the sole carbon source. A stock culture was obtained after 36 h incubation in a rotatory shaker at 28°C.

Biodegradation assays were performed in a 1,250 mL effective volume microfermentor (New Brunswick Multigen TA). The reactor was aerobically operated at 28°C. The same minimal medium used in the adaptation stage was employed, supplemented with ibuprofen as the carbon source. The medium was inoculated with 5 mL of the stock culture (2×10^8 CFU mL⁻¹). In order to monitor the biodegradation process, 10 mL samples were removed from the microfermentor at appropriate intervals (usually 3 h) and both ibuprofen concentration and bacterial growth were measured. Triplicates of each assay were carried out. The abiotic loss of the compound was estimated in a control assay without inoculation.

Biodegradation test in fixed-bed reactor

Continuous biodegradation assays were performed in an aerobic down-flow fixed-bed reactor constructed from polyvinyl chloride (PVC, 100 cm \times 10 cm internal diameter). A more detailed description of the reactor was recently published.[46] The column was filled with hollow cylinders, approximately 15 mm \times 15 mm in diameter, employed as a support for immobilizing the bacterial biofilm. Cylinders were cut from PVC conduit usually employed for electrical wiring.

Before start-up, the PVC cylinders were first inoculated with the bacterial community in an aerobic batch reactor with an effective volume of 1 L and successively fed in batch mode with 50 mg L⁻¹ of ibuprofen in order to allow biofilm development. The reactor was operated for one month in fed-batch mode and then the cylinders were transferred to the column.

The reactor was continuously fed with synthetic wastewater prepared by dissolving ibuprofen, 100 mg L^{-1} , in free chlorine tap water supplemented with a farming fertilizer (BASF), ratio N:P:K about 10:2:6. It was operated under environmental conditions: without sterility and at room temperature. The influent flow rate was $1 L \text{ day}^{-1}$. A similar reactor without inoculation was operated to estimate abiotic losses of ibuprofen.

Biofilm development on PVC cylinders was observed by scanning electronic microscopy (SEM) (Philips XI30). Samples for SEM analysis were taken from the reactor and fixed as previously described.[33]

Analytical methods and control parameters

To determine the amount of remaining ibuprofen, bacterial cells were separated by centrifugation, and the filtered supernatant fluid was submitted to spectrophotometrical analysis (Metrolab UV 1700 Spectrophotometer); the absorbance was measured at 220 nm. To prove complete degradation of ibuprofen, selected samples taken at the end of the degradation process were also determined by HPLC as described in the following paragraph. COD was measured in samples taken at the beginning and at the end of the batch process, and at the influent and effluent of the continuous reactor, according to APHA.[32]

Determination of cell viability in batch and continuous reactors was performed by spreading sample dilutions on the surface of TSA plates.[32] To estimate the number of bacteria attached to the PVC cylinders, 10 g of the material was aseptically suspended in 100 mL of sterile saline solution and subjected to vortex agitation for 4 min. The counting and identification of the removed bacteria from the reactor support were then carried out.

Instrumentation and chromatographic conditions

The Spectra System HPLC equipment assembled comprised a Thermo Scientific SCM1000 Quaternary pump, P4000 degasser, AS3000 autosampler and UV2000 Dual λ Absorbance detector. Analysis of chromatograms was carried out using a ChromQuest Chromatography Data System software. Separation of ibuprofen was achieved with a Spherisorb ODS 2 column (150 mm × 4.6 mm i.d 5 µm).

The chromatographic condition was as follows: an injection volume of 10 μ L, a column temperature of 25°

C with an isocratic mobile phase prepared with a mixture of 0.01 M phosphoric acid: acetonitrile (50:50, v v⁻¹) and a flow rate of 2.0 mL min⁻¹. UV-detection was performed at λ 214 nm. Separation of ibuprofen was accomplished in less than 6 min.

Results

Biodegradability of ibuprofen in surface waters

Water from six points of the basin was analyzed; two of them located in the upper basin (Rodríguez stream and Morales stream), one in the middle (Ricchieri Highway Bridge) and three in the bottom of the basin, near its mouth on the Rio de la Plata (La Noria Bridge, Alsina Bridge and La Boca). The location of the sampling points is shown in Figure 1. Characterization of water samples is shown in Table 1. The results showed that under the conditions of the assay, ibuprofen was biodegraded in four of the six points tested (Figure 2). In all cases, biodegradation was confirmed in batch assays in mineral minimal medium with the compound as the sole carbon source. In those tests, the sample from La Noria Bridge showed the highest degradative capacity and was selected to perform the subsequent assays (data not shown).

Selection and identification of ibuprofendegrading bacteria

Studies conducted have shown that ibuprofen degradation was carried out by a bacterial community composed of two bacteria. None of the individual strains were capable of degrading the compound as the sole



Figure 1. Location of sampling points in the Matanza-Riachuelo basin: 1, Rodríguez stream; 2, Morales stream; 3, Ricchieri Bridge; 4, La Noria Bridge; 5, Alsina Bridge; 6, La Boca.

Tak	ole	1.	Charac	terization	of	surface	water	sampl	es.
-----	-----	----	--------	------------	----	---------	-------	-------	-----

			Heterotrophic			
Sampling point	$\begin{array}{c} \text{COD} \\ \text{(mg } \text{L}^{-1} \text{ O}_2 \text{)} \end{array}$	$\begin{array}{c} \text{BOD} \\ \text{(mg } L^{-1} \text{ O}_2 \text{)} \end{array}$	plate count (CFU mL ⁻¹)	<i>E. coli</i> count	Enterococcus count	
Rodríguez	29	10	1.0×10^{4}	$3.0^{a} \times 10^{3}$	$4.6^{a} \times 10^{2}$	
Morales stream	39	18	1.5×10^{4}	$1.1^{a} \times 10^{3}$	$1.1^{a} \times 10^{3}$	
Ricchieri Bridge	89	11	6.5×10^{4}	$1.9^{b} \times 10^{2}$	$2.4^{a} \times 10^{2}$	
La Noria Bridge	90	28	1.8×10^{6}	$4.4^{b} \times 10^{3}$	$4.5^{b} \times 10^{3}$	
Alsina Bridge	90	23	9.3×10^{5}	$9.5^{b} \times 10^{3}$	$7.5^{b} \times 10^{3}$	
La Boca	98	15	1.9×10^{5}	$9.5^{b} \times 10^{3}$	$1.1^{b} \times 10^{3}$	
- 1						

^aMPN 100 mL⁻¹.

^bCFU mL⁻¹.



Figure 2. Oxygen consumption in surface water samples: control (-o-) and with 20 mg L⁻¹ of ibuprofen $(-\blacksquare-)$. 1, Rodríguez stream; 2, Morales stream; 3, Ricchieri Bridge; 4, La Noria Bridge; 5, Alsina Bridge; 6, La Boca.

carbon source. Owing to this inability, tests were carried out with the bacterial community. The partial 16S rRNA gene sequence showed that the indigenous bacterial community consisted of *Comamonas aquatica* and *Bacillus* sp. Identity percentage obtained was, respectively, 100% and 99%. The bacterial community proved to be stable during all the months in which the experiments were conducted.

Biodegradation assay in batch reactor

Biodegradation assays were carried out with an initial inoculum of 1×10^6 cells mL⁻¹. The assays showed that the indigenous community was capable of degrading 100 mg L⁻¹ of ibuprofen in 33 h, with a lag phase of 3 h (Figure 3). The specific growth rate (μ) was 0.21 h⁻¹. The removal of the compound exceeded 99% of the initial concentration, with 92.3% removal of COD. The control assay shows the absence of abiotic loss in the



Figure. 3. Degradation of ibuprofen by the bacterial community in batch reactor.

system. Complete degradation of ibuprofen was proved by HPLC performed at the end of the batch process (Figure 4).



Figure 4. HPLC chromatograms: (A) initial batch process – continuous reactor influent; (B) final batch process; and (C) continuous reactor effluent.

Biodegradation test in fixed-bed reactor

The down-flow fixed-bed reactor shows an average removal efficiency of 95.9% of ibuprofen and 92.3% of COD. The average inlet concentration was 110.4 mg L⁻¹ and the maximal removal rate for the compound was 17.4 g m⁻³d⁻¹. Process efficiency remained approximately constant regardless of the fluctuation in temperature (17.5–31.4°C) during the 70 days that the reactor was maintained in operation (Figure 5). The absence of abiotic loss in the system was proved by the control assay carried out simultaneously. Re-inoculation was not necessary; the biomass of the community developed from the initial inoculum. The continuous reactor was



Figure 5. Degradation of ibuprofen by the bacterial community in aerobic down-flow fixed-bed reactor. Ibuprofen concentration (mg L^{-1}) in the influent ($-\blacksquare$ —) and in the effluent ($-\blacksquare$ —).

operated without sterility, and proves the ability of the bacterial community to grow and degrade the compound in a natural environment. The removal efficiency achieved was similar to that obtained in minimal medium at regulated pH and temperature, and in the absence of other microorganisms.

Total bacterial count in the reactor effluent reached 2.6×10^6 CFU mL⁻¹, whereas remotion by vortex agitation allowed us to estimate a biomass of 2.3×10^9 CFU g⁻¹ in the support material. Both *Comamonas aquatica* and *Bacillus* sp. were isolated from the reactor effluent and from the PVC cylinders. Although other environmental bacteria were isolated from these samples, none were able to degrade ibuprofen when they were assayed to prove compound degradation in the same way as previously described for community constituents. As in the batch reactor, HPLC results (Figure 4) associated with the decrease in COD values and the absence of UV absorbance demonstrated the mineralization of the compound and the absence of metabolites.

Biofilm development in the PVC cylinder surface can be seen in the micrographs obtained from scanning electron microscopy (Figure 6).

Discussion

The Matanza-Riachuelo basin is one of the most polluted in Argentina, which extends throughout Buenos Aires City and the bordering province of Buenos Aires. The basin population exceeds 4,800,000 inhabitants, 65% of whom lack sewer systems. Moreover, daily industrial dumping in the basin was estimated by Autoridad de Cuenca Matanza-Riachuelo, the basin Authority, at 88,500 m³ in 2003 (www.acumar.gov.ar). In such a polluted environment, it is to be expected that bacterial



Figure 6. Scanning electron photomicrographs of PVC cylinders surface: before inoculation (A) 50×; and after inoculation (B) 1000× and (C) 3500×.

pre-exposition to persistent compounds facilitates the selection of biodegradative strains. Although it was desirable, we were not able to detect ibuprofen in the surface waters. But it is well known that the main source of pharmaceuticals is the sewage waters.[2,15] So we intended correlate the presence of degrading bacteria with sewage pollution. However, the presence of ibuprofendegrading bacterial communities does not appear to correlate only with pollution. In the sampling carried out, degrading bacteria were isolated from rural relatively unpolluted areas, such as the stream Rodriguez, while in sampling points such as the Riachuelo in the area of La Boca, a paradigm of pollution in our country, with Escherichia coli count values close to $1 \times 10^4 \text{ mL}^{-1}$, we not able to isolate ibuprofen-degrading were communities.

The presence of degrading bacteria in the samples without oxygen consumption above the control cannot be excluded. It should be noted that the assay term is relatively short and is conducted without a prior compound adaptation step; but the presence of degrading bacteria could not be correlated with sewage pollution, probably the most important source of ibuprofen in the environment. Anyway the fact that degrading communities were found in most areas agrees with those reported in the literature, which indicates that compared to other drugs, ibuprofen is relatively biodegradable. Matamoros et al. [34] report values of 60-70% from remotion in conventional treatment plants. However, biodegradability reports vary widely. In a compilation of the results of 125 activated sludge-based treatment plant systems, removal efficiencies of ibuprofen vary between 40% and 100%.[35] Despite this, ibuprofen is still considered a priority compound within the context of pharmaceuticals. This is due to the predicted environmental values and its potential toxic effect.[36-38]

Physicochemical treatments have been proposed for the treatment of effluents containing ibuprofen. Without ignoring the versatility that these processes present, they can hardly compete in terms of cost with biological processes when a single chemical species is involved. Méndez-Arriaga et al. [39] obtained by photo-Fenton degradation between 25% and 60% for an initial concentration of ibuprofen of the same order as presented in this work.

The isolated bacterial community consisted of *Comamonas aquatica* and *Bacillus* sp., two genera within which many strains have been described related to the degradation of persistent compounds (The Biodegradative Strain Database http://bsd.cme.msu.edu/BSD). Degradation of a compound by a community in which none of its members separately is capable of degrading it has been already described.[40] Even we have had similar experiences in our laboratory.[41] Like what happened in those studies, the possibility that ibuprofen was degraded by another strain which does not grow in the solid medium used cannot be rejected.

As expected according to data reported in the literature,[42, 43] abiotic losses were not obtained during the experiments.

There are few studies on the isolation of bacteria or bacterial communities capable of using ibuprofen as the sole carbon source. Murdoch and Hay [44] have isolated a strain of *Sphingomonas* for which the degradative pathway has also been established. There are also no application data of isolated indigenous bacteria or communities for the continuous treatment of effluents containing ibuprofen. In a recent study conducted, Tiehm et al. [45] studied ibuprofen biodegradation in batch reactors and sand columns. Similarly, in another study Tadkaew et al. [46] employed a laboratory-scale membrane bioreactor to degrade the compound. In both cases, the efficiency achieved was similar to that of the present study, but the inoculum used was sludge from municipal wastewater treatment plants.

A wide variety of materials have been employed for supporting growth in biofilm reactors. The most frequently used include calcium alginate,[47] granulated activated carbon [48] and polyurethane foam.[49] Qureshi et al. [50] employed hollow PVC cylinders cut from a conduit used in electrical wiring in a biofilm reactor for copper adsorption. In a previous work, we used the same support material for the treatment of 2,4-dichlorophenol and 2,4,6-trichlorophenol.[51]

In order to promote biomass development in the support material and thus facilitate the start-up of the reactor, it was operated previously in fed-batch mode as reported elsewhere.[52–54] This objective was reached, since ibuprofen concentration in the effluent decreased to average minimum values in the first days of operation.

Conclusions

We have found that samples from four of the six points tested in the Matanza-Riachuelo basin were able to degrade ibuprofen. This does not seem to correlate with fecal contamination in the watershed. The community isolated from La Noria Bridge showed the highest degradation capability and consisted of *Comamonas aquatica* and *Bacillus* sp. The degradation efficiency for an initial concentration of 100 mg L⁻¹ exceeds 92% of COD both in batch and continuous reactors.

Effluents of the pharmaceutical industry are a source of pollution with pharmaceuticals whose importance should not be minimized. Being unregulated contaminants, an industry can comply perfectly with the values of contaminants allowed in effluent by legislation and still be eliminating a high concentration, in environmental terms, of these pollutants. For these cases, the treatment of partial effluent streams before reaching the main treatment plant may be a solution. The proposed reactor could be an alternative to fulfill this purpose. One of the objectives of our work is transferring results to industrial applications; hence, operating and construction costs play an important role in reactor design. Both the use of an inexpensive support material and the lack of aeration costs are desirable for such purposes. It is also advantageous to be able to work without temperature control or sterility.

It is suggested that the ability of the isolated indigenous community can be exploited to improve the treatment of wastewaters containing ibuprofen. The use of the continuous reactor proposed in this work can be considered as an inexpensive alternative to treat effluents efficiently.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Universidad de Buenos Aires under grant UBACYT Program – Project 20020100100822 (2011–2014) and Project 20020130100378 BA (2014–2017).

References

- [1] Kuster M, Alda M, Hernando M, Petrovic M, Martín A, Barceló D. Analysis and occurrence of pharmaceuticals, estrogens, progestogens and polar pesticides in sewage treatment plant effluents, river water and drinking water in the Llobregat river basin (Barcelona, Spain). J Hydrol. 2008;358:112–123.
- [2] Jones OAH, Voulvoulis N, Lester JN. Human pharmaceuticals in the aquatic environment a review. Environ Technol. 2001;22:1383–1394.
- [3] Clara M, Strenn B, Gans O, et al. Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants. Water Res. 2005;39:4797– 4807.
- [4] Kümmerer K. Resistance in the environment. J Antimic Chemother. 2004;54:311–320.
- [5] Gagné F, Blaise C, André C. Occurrence of pharmaceutical products in a municipal effluent and toxicity to rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Ecotoxicol Environ Saf. 2006;64:329–336.
- [6] Lindqvist N, Tuhkanen T, Kronberg L. Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters. Tampere, Turku, Finland. Water Res. 2005;39:2219–2228.
- [7] Ferrari B, Paxéus N, Lo Giudice R, et al. Ecotoxicological impact of pharmaceuticals found in treated wastewaters: study of carbamazepine, clofibric acid, and diclofenac. Ecotoxicol Environ Saf. 2003;55:359–370.
- [8] Carballa M, Omil F, Lema JM, et al. Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. Water Res. 2004;38:2918–2926.
- [9] Gómez MJ, Martínez Bueno MJ, Lacorte S, et al. Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast. Chemosphere. 2007;66:993–1002.
- [10] Kosjek T, Heath E, Pérez S, et al. Metabolism studies of diclofenac and clofibric acid in activated sludge bioreactors using liquid chromatography with quadrupole – time-of-flight mass spectrometry. J Hydrol. 2009;372:109–117.
- [11] Bendz D, Paxéus NA, Ginn TR, et al. Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Höje River in Sweden. J Hazard Mater. 2005;122:195–204.
- [12] Joss A, Zabczynski S, Göbel A, et al. Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing a classification scheme. Water Res. 2006;40:1686–1696.
- [13] Tauxe-Wuersch A, De Alencastro LF, Grandjean D, et al. Occurrence of several acidic drugs in sewage treatment plants in Switzerland and risk assessment. Water Res. 2005;39:1761–1772.
- [14] Jones OAH, Voulvoulis N, Lester JN. The occurrence and removal of selected pharmaceutical compounds in a

sewage treatment works utilizing activated sludge treatment. Environ Pollut. 2007;145:738–744.

- [15] Halling-Sørensen B, Nielsen SN, Lanzky PF, et al. Occurrence, fate and effects of pharmaceutical substances in the environment – a review. Chemosphere. 1998;36:357–393.
- [16] Heberer T. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol Lett. 2002;131:5–17.
- [17] Gros M, Petrovic M, Ginebreda A. Removal of pharmaceuticals during wastewater treatment and environemntal risk assessment using hazard indexes. Environ Int. 2010;36:15–26.
- [18] Daughton CG, Ternes TA. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ Health Perspect. 1999;107:907–938.
- [19] Radjenovic J, Petrovic M, Barceló D. Analysis of pharmaceuticals in wastewater and removal using a membrane bioreactor. Anal Bioanal Chem. 2007;387:1365–1377.
- [20] Ternes TA. Occurrence of drugs in German sewage treatment plants and rivers. Water Res. 1998;32:3245–3260.
- [21] Hirsch RU, Ternes T, Haberer K, et al. Occurrence of antibiotics in the aquatic environment. Sci Total Environ. 1999;225:109–118.
- [22] Badawy MI, Wahaab RA, El-Kalliny AS. Fenton-biological treatment processes for the removal of some pharmaceuticals from industrial wastewater. J Hazard Mater. 2009;167:567–574.
- [23] Panorel I, Preis S, Kornev I, et al. Oxidation of aqueous pharmaceuticals by pulsed corona discharge. Environ Technol. 2013;34:923–930.
- [24] Dougherty J, Swarzenski P, Dinicola R, et al. Occurrence of herbicides and pharmaceutical and personal care products in surface water and groundwater around Liberty Bay, Puget Sound, Washington. J Environ Qual. 2010;39:1173–1180.
- [25] Winkler M, Lawrence JR, Neu TR. Selective degradation of ibuprofen and clofibric acid in two model river biofilm systems. Water Res. 2001;35:3197–3205.
- [26] Bedne M, MacCrehan WA. Transformation of acetaminophen by chlorination produces the toxicants 1,4-benzoquinone and *N*-acetyl-*p*-benzoquinone imine. Environ Sci Technol. 2006;40:516–522.
- [27] Vieno N, Tuhkanen T, Kronberg L. Removal of pharmaceuticals in drinking water treatment: effect of chemical coagulation. Environ Technol. 2006;27:183–192.
- [28] Fent K, Weston AA, Caminada D. Ecotoxicology of human pharmaceuticals. Aquat Toxicol. 2006;76:122–159.
- [29] Cleuvers M. Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid. Ecotoxic Environ Saf. 2004;59:309–315.
- [30] Kümmerer K, Al-Ahmad A, Mersch-Sundermann V. Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test. Chemosphere. 2000;40:701–710.
- [31] Kupe L, Schanz F, Bachofen R. Biodiversity in the benthic diatom community in the upper River Töss reflected in water quality indices. CLEAN – Soil Air Water. 2008;36:84–91.
- [32] American Public Health Association, American Water Works Association, Water Environment Federation. Standard methods for the examination of water and wastewater. 22nd ed. Washington, DC: American Public Health Association; 2012.

- [33] González AJ, Gallego A, Gemini VL, et al. Degradation and detoxification of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) by an indigenous *Delftia* sp. strain in batch and continuous systems. Int Biodeter Biodegrad. 2012;66:8–13.
- [34] Matamoros V, García J, Bayona JM. Organic micropollutant removal in a full-scale surface flow constructed wetland fed with secondary effluent. Water Res. 2008;42:653–660.
- [35] Miège C, Choubert JM, Ribeiro L, et al. Fate of pharmaceuticals and personal care products in wastewater treatment plants – conception of a database and first results. Environ Pollut. 2009;157:1721–1726.
- [36] Besse JP, Garric J. Human pharmaceuticals in surface waters implementation of a prioritization methodology and application to the French situation. Toxicol Lett. 2008;176:104–123.
- [37] Zhang D, Luo J, Ping Lee ZM, Gersberg RM, et al. Ibuprofen removal in horizontal subsurface flow constructed wetlands: treatment performance and fungal community dynamics. Environ Technol. Forthcoming 2016. doi:10. 1080/09593330.2015.1119201
- [38] Hashim NH, Shafie S, Khan SJ. Enantiomeric fraction as an indicator of pharmaceutical biotransformation during wastewater treatment and in the environment – a review. Environ Technol. 2010;31:1349–1370.
- [39] Méndez-Arriaga F, Esplugas S, Giménez J. Degradation of the emerging contaminant ibuprofen in water by photo-Fenton. Water Res. 2010;44:589–595.
- [40] Kharoune L, Kharoune M, Lebeault JM. Aerobic degradation of 2,4,6-trichlorophenol by a microbial consortium – selection and characterization of microbial consortium. Appl Environ Microbiol. 2002;59:112–117.
- [41] Gallego A, Gemini VL, Rossi S, et al. Detoxification of 2,4,6trichlorophenol by an indigenous bacterial community. Int Biodeter Biodegrad. 2009;63:1073–1078.
- [42] Yamamoto H, Nakamura Y, Moriguchi S, et al. Persistence and partitioning of eight selected pharmaceuticals in the aquatic environment: laboratory photolysis, biodegradation, and sorption experiments. Water Res. 2009;43:351– 362.
- [43] Yu TH, Lin AYC, Panchangam SC, et al. Biodegradation and bio-sorption of antibiotics and non-steroidal anti-inflammatory drugs using immobilized cell process. Chemosphere. 2011;84:1216–1222.
- [44] Murdoch RM, Hay AG. Formation of catechols via removal of acid side chains from ibuprofen and related aromatic acids. Appl Environ Microbiol. 2005;71:6121–6125.
- [45] Tiehm A, Schmidt N, Stieber M, et al. Biodegradation of pharmaceutical compounds and their occurrence in the Jordan valley. Water Resour Manag. 2011;25:1195–1203.
- [46] Tadkaew N, Hai FI, McDonald JA, et al. Removal of trace organics by MBR treatment: the role of molecular properties. Water Res. 2011;45:2439–2451.
- [47] González G, Herrera G, García MT, et al. Biodegradation of phenolic industrial wastewater in a fluidized bed bioreactor with immobilized cells of *Pseudomonas putida*. Bioresour Technol. 2001;80:137–142.
- [48] Wilson GJ, Khodadoust AP, Suidan MT, Brenner RC, Acheson CM. Anaerobic/aerobic biodegradation of pentachlorophenol using GAC fluidized bed reactors: optimization of the empty bed contact time. Water Sci Technol. 1998;36(6):107–115.

- 10 👄 M. S. FORTUNATO ET AL.
- [49] Hsien TY, Lin YH. Biodegradation of phenolic wastewater in a fixed biofilm reactor. Biochem Eng J. 2005;27:95–103.
- [50] Qureshi FM, Badar U, Ahmed N. Biosorption of copper by a bacterial biofilm on a flexible polyvinyl chloride conduit. Appl Environ Microbiol. 2001;67:4349–4352.
- [51] Gallego A, Gemini VL, Rossi SL, et al. Degradation and detoxification of chlorophenols in continuous-flow fixedbed aerobic reactors. CLEAN – Soil Air Water. 2011;39:774–780.
- [52] Sá CSA, Boaventura RAR. Biodegradation of phenol by *Pseudomonas putida* DSM 548 in a trickling bed reactor. Biochem Eng J. 2001;9:211–219.
- [53] Quan X, Shi H, Zhang Y, et al. Biodegradation of 2,4dichlorophenol in an air-lift honeycomb-like ceramic reactor. Process Biochem. 2003;38:1545–1551.
- [54] Kargi F, Eker S. Removal of 2,4-dichlorophenol and toxicity from synthetic wastewater in a rotating perforated tube biofilm reactor. Process Biochem. 2005;40:2105–2111.