Front. Environ. Sci. Eng. 2018, 12(5): 15
https://doi.org/10.1007/s11783-018-1080-5

RESEARCH ARTICLE

Isolation and application of an ibuprofen-degrading bacterium to a biological aerated filter for the treatment of micro-polluted water

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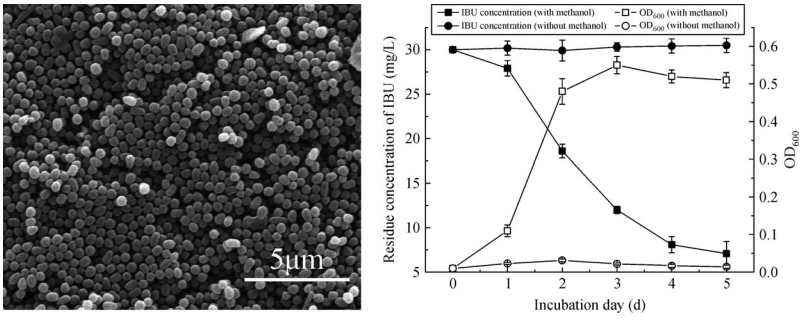
HIGHLIGHTS

- An ibuprofen-degrading strain, *Serratia marcescens* BL1, was isolated and identified.
- The effects of various factors on ibuprofen degradation by BL1 were evaluated.
- Strain BL1 was applied to a laboratory-scale biological aerated filter system.
- Strain BL1 was stable in both static tests and in the biological aerated filter system.

GRAPHIC ABSTRACT



ARTICLE INFO

Article history:
Received 19 January 2018
Revised 20 July 2018
Accepted 20 July 2018
Available online 18 September 2018

Keywords:
Ibuprofen
Biological aerated filter
Degrading bacterium
Serratia marcescens

ABSTRACT

Ibuprofen (IBU) is widely used in the world as anti-inflammatory drug, which posed health risk to the environment. A bacterium capable of degrading IBU was isolated from activated sludge in a sewage treatment plant. According to its morphological, physiologic, and biochemical characteristics, as well as 16S rRNA sequence analysis, the strain was identified as *Serratia marcescens* BL1 (BL1). Degradation of IBU required the presence of primary substrate. After a five-day cultivation with yeast powder at 30°C and pH 7, the highest degradation (93.47%±2.37%) was achieved. The process of BL1 degrading IBU followed first-order reaction kinetics. The BL1 strain was applied to a small biological aerated filter (BAF) device to form a biofilm with activated sludge. IBU removal by the BAF was consistent with the results of static tests. The removal of IBU was 32.01% to 44.04% higher than for a BAF without BL1. The indigenous bacterial community was able to effectively remove COD_{Mn} (permanganate index) and ammonia nitrogen in the presence of BL1.

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1 Introduction

Ibuprofen (IBU) is an anti-inflammatory, analgesic, and antipyretic drug that is widely used in the treatment of migraines, rheumatoid arthritis, and neuritis. IBU is the third most commonly used drug in the world (Ali et al., 2009), and the annual production of IBU is in the thousands of tons (Ali et al., 2009; Paíga et al., 2013). After IBU is administered and undergoes hepatic metabolism, 15% of ibuprofen is excreted unaltered. Its metabolites, 2-OH-IBU and CBX-IBU, account for 26% and 43% of total ibuprofen ingested, respectively (Ferrando-Climent et al., 2012). Those metabolites can enter city sewage treatment plants or natural water bodies directly. These substances are degraded into small organic compounds that are adsorbed onto the surface of sludge

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and converted into lipophilic substances. They may also exist in their original forms (Halling-sørensen et al., 1998). The two metabolites enter surface waters with the effluent of sewage treatment plants, and they can persist and accumulate in the environment (Ferrando-Climent et al., 2012). IBU has been detected in rivers and lakes in several countries throughout the world at concentrations ranging from 5 ng/L to 1.5 μg/L (Tran et al., 2014a; 2014b; Phan et al., 2015; Lindholm-Lehto et al., 2016; Zhao et al., 2016), and the health risk this micro-polluted water poses to aquatic organisms and humans cannot be ignored (Chu et al., 2010; Han et al., 2010;Parolini et al., 2011; Pietrini et al., 2015;). Studies have shown that IBU and its metabolites can affect the physiologic processes of several species (Han et al., 2010; Parolini et al., 2011). Therefore, the removal of IBU residues is urgent in water treatment systems. Although IBU has become a notable water pollutant because of its high consumption and high detection rate (de Sousa et al., 2014), the monitoring levels and conditions vary from region to region in China. It is difficult to effectively pretreat and detect trace contaminants in some areas. Therefore, to ensure safe drinking water, there is a need to improve decontamination strategies for micro-polluted water. The efficient removal of IBU can be achieved by modifying or adjusting the operating conditions of the original treatment systems.

Biological pretreatment, conventional physical and chemical treatments, and advanced treatments are used in water treatment facilities to meet water quality standards. Due to the continuous deterioration of water source quality, biological pretreatment is used as a complementary technique with conventional treatment to effectively remove dissolved organic matter and reduce water mutagenicity (Qim et al., 2007; Wang et al., 2014). Biological pretreatment methods include biological contact oxidation and biological aerated filters (BAF). Biological contact oxidation is a traditional method that has been applied in many water treatment plants, while BAF has been promoted in recent years due to its small footprint, promotion of stable water quality, low operating costs, low infrastructure costs, and other advantages. In BAF, the biofilm on the filter surface is the main source of the metabolism of organic matter. Satisfactory results have been achieved in some studies using a combination of electrodes and BAF for the treatment of micro-polluted source water (He et al., 2014; Baghapour et al., 2015; Gao et al., 2015). Currently, IBU-degrading microorganisms include *Spirochaetaceae*, *Sphingomonas*, and *Bacillus thuringiensis* (Murdoch and Hay, 2013; Li et al., 2016; Marchlewicz et al., 2017), whose IBU degradation efficiencies are less than 50% under normal operating conditions. In this study, a new IBU-degrading bacterium, *Serratia marcescens*, was isolated and is being reported for the first time. This bacterium demonstrates the ability to degrade IBU more efficiently. *Serratia marcescens* is capable of degrading persistent organic pollutants in soils (Cycoń et al., 2013), but to the best of our knowledge, no study has reported its degradation performance of pollutants in water. In this study, *Serratia marcescens* was applied to a BAF to investigate its IBU degradation ability.

2 Materials and methods

2.1 Materials

IBU (>98%) and methanol (high-performance liquid chromatography (HPLC) grade) were purchased from Sigma (Saint Louis, USA) and Honeywell (Shanghai, China), respectively. The remaining chemicals were of analytical grade and purchased from the Sinopharm Group (Shanghai, China). The inorganic salt medium was composed of 4.35 mg/L K_2HPO_4, 1.7 mg/L KH_2PO_4, 2.1 mg/L NH_4C1, 0.2 mg/L $MgSO_4$, 0.05 mg/L $MnSO_4$, 0.01 mg/L $FeSO_4 \cdot 7H_2O$, and 0.03 mg/L $CaC1_2 \cdot 2H_2O$ (pH 6.8–7.0). The nutrient broth medium consisted of 3 mg/L beef extract, 10 mg/L peptone, and 5 mg/L NaCl (pH 7.0–7.2). Nutrient agar solid medium was composed of 3 mg/L beef extract, 10 mg/L peptone, 5 mg/L NaCl, and 15 mg/L agar powder (pH 7.0–7.2).

2.2 Enrichment and isolation of IBU-degrading bacteria

To 100 mL of autoclaved inorganic salt medium containing 1 mg of IBU and 1 mL of methanol, 2 g of sludge sample from the Shanghai Songjiang Sewage Treatment Plant were added, and the culture was incubated in a rotary shaker (DKY-II, Duke Automatic Equipment Company, Shanghai, China) at 30°C and 160 r/min in the dark. After three days, the above culture was inoculated into fresh medium at an inoculum concentration of 10%, and the procedure was repeated once. The centrifuged cells were inoculated in the same manner as described above, and the contents of IBU in the media were 1.5, 2.0, 2.5, 3.0, and 3.5 mg. Subsequently, the cultures were diluted 10^6 to 10^9 times before they were plated, and cultivation was performed at 30°C for 24 h. Colonies with different morphological characteristics were rescreened several times to obtain single colonies with the same morphology. The colonies were inoculated into broth culture medium for expansion. The obtained cells were washed three times using sterile saline and were inoculated into the inorganic salt medium. After three days, culture broths were centrifuged at 8000 r/min for 15 min at 4°C. Then the supernatants were filtered through a membrane filter of 0.45 μm pore size, and IBU residues were measured by high performance liquid chromatography (HPLC) (Agilent 1100, USA). Each isolated bacterium was cultivated and tested in triplicate, and the average value of residual IBU

was calculated. The average values were compared among all isolated bacteria to select the optimal IBU-degrading strain.

2.3 Characterization of the isolated strain

Bacterial colonies and the morphology of isolated individual bacteria were characterized. Experiments to identify the bacterial species were performed according to the methods of Shen and Chen (2007), including Gram stain reaction, glucose gas production test, starch hydrolysis test, sucrose fermentation test, methyl red test, hydrogen sulfide production test, and catalase test.

The morphology of the isolates was analyzed by scanning electron microscopy (SEM). Fresh bacterial liquid at the logarithmic growth phase was collected and fixed with 2.5% glutaraldehyde for 2 h. The bacterial cells were collected using centrifugation, washed by phosphate buffer and sterile water repeatedly, and freeze-dried. Samples were observed using a JSM-5600LV SEM (JEOL, Japan) at the Test Center of Donghua University.

2.4 Identification of the isolated strain

16S rRNA gene sequencing of the isolated strain was performed by Sangon Biotech (Shanghai, China). Genomic DNA was extracted from the strain using a SK1201-UNIQ-10 column bacterial genomic DNA extraction kit (Sangon) following the manufacturer's standard protocol. The genomic DNA was used as a target for PCR amplification using primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The PCR products were subjected to agarose gel electrophoresis and were purified using a SK1131 UNIQ-10 column DNA gel extraction kit (Sangon). The gene sequence obtained from the above process was compared with other bacterial sequences using GenBank (http://www.ncbi.nlm.nih.gov/BLAST/).

2.5 Degradation analysis of IBU-degrading bacteria

2.5.1 IBU measurement

The HPLC operating conditions were as follows: Agilent ZORBAX Eclipse XDB-C18 column (150 mm × 4.6 mm, 5 μm), water (pH adjusted to 3 by phosphoric acid) and acetonitrile (50:50, V/V) mobile phase, 1.0 mL/min flow rate, 222 nm detection wavelength, and 20 μL injection volume. For solid-phase extraction (SPE), 1000 mL of water sample was filtered through a 0.45-μm filter, and the pH was adjusted to 3 with hydrochloric acid. C18 was used as the adsorbent in the SPE column. The sample loading flow rate was 5 mL/min; the eluent was 95% methanol in water solution; and a stepwise elution was performed (2 mL, 2 mL, and 3 mL). The eluate was dried under nitrogen flow, and then 1 mL methanol was added to the sample bottle to dissolve dried eluate. The detection limit of IBU by HPLC was 0.040 μg/L.

2.5.2 Parameters affecting IBU degradation by strain BL1

Yeast powder, peptone, and glucose (5 g/L) were added to the inorganic salt medium to examine the effect of nutrients on the degradation of IBU. Yeast powder (5 g/L) was used as the primary substrate in the inorganic salt medium to investigate the effect of pH and temperature on IBU degradation. The pH was adjusted by 0.1 mol/L hydrochloric acid and 0.1 mol/L sodium hydroxide, and the culture temperature was adjusted by the temperature control device in the shaker. Strain growth (OD_{600}) was measured at 600 nm by a UV/Vis spectrophotometer (TU-1900, Persee, China). Each sample measurement was carried out in triplicate, and the results are expressed as the mean values.

2.6 Application of strain BL1 to a BAF system

Two BAF systems (B1 and B2) were used in this study. The inner diameter of the filter was 100 mm. There was a water inlet and a backwash water outlet located on the top, and a water outlet, a backwash water intake, and air inlet located at the bottom. The filling was ceramsite with a height of 1000 mm, and downstream flow mode was used for operation. Biofilm was formed in B1 using sludge from the Sewage Treatment Plant of Songjiang Shanghai. An extra 400 mL of BL1 strain (cultured for one day) was added for biofilm formation in B2. The nutrient solution composition for biofilm formation was as follows: 100 mg/L glucose, 18.86 mg/L NH_4SO_4, 3.51 mg/L KH_2PO_4, 0.61 mg/L $CaCl_2$, and 2.87 mg/L $MgSO_4$. During the 10 d formation period, the removal efficiency of permanganate index (COD_{Mn}) and ammonia nitrogen (NH_3-N) was stably above 10% and 30%, respectively; thus the BAF was well formatted. Concentrations of COD_{Mn} and NH_3-N were measured as described in the standard methods of the Ministry of Environmental Protection of the People's Republic of China (1989; 2009). After the successful formation of the biofilm, the influent water was changed to micro-polluted water, which was prepared with water from Jingyue Lake at Donghua University and 10 μg/L of IBU. The influent water temperature was between 15.6°C and 24.5°C, with pH of 6.88–7.27, COD_{Mn} of 5.4–8.6 mg/L, and NH_3-N of 1.25–2.85 mg/L. The ratio of gas to water was 1.5:1, and dissolved oxygen (DO) at the top of the BAF was maintained at 5 mg/L. The hydraulic load was 0.2 $m^3/(m^2 \cdot h)$. A gas-water combined backwash was performed, with an operation cycle of 7–8 d, a duration of 8–10 min, a water washing intensity of 7–8 $L/(m^2 \cdot s)$, and an air washing intensity of 3–4 $L/(m^2 \cdot s)$.

3 Results and discussion

3.1 Isolation

After domestication and isolation, five strains grew well in culture broths. Two of the strains, BL2 and BL3, exhibited IBU tolerance, but they could not degrade IBU or use it as an energy source. Only three strains showed the ability to remove IBU: BL1, BL4, and BL5. The culture broths of these three strains appeared canary yellow and milk white. The OD_{600} of BL1 (0.52) was higher than that of BL2 (0.40) and BL3 (0.33) on day 3. According to their IBU removal abilities on day 6 (59.6%±1.18%, 55.1%±0.98%, and 39.7%±1.31%, respectively), BL1 was selected for follow-up experiments.

3.2 Strain identification

The BL1 colony was circular in shape with a diameter of 1.5–2.0 mm (Fig. 1). It was red in color and opaque, with regular edges, a smooth surface, and a granular center. As for its physical and chemical characteristics, the results of Gram staining, the starch hydrolysis test, methyl red test, and hydrogen sulfide production test were all negative. The results of glucose gas production, sucrose fermentation, and catalase tests were all positive, and the aerobic test showed that BL1 was a facultative bacterium.

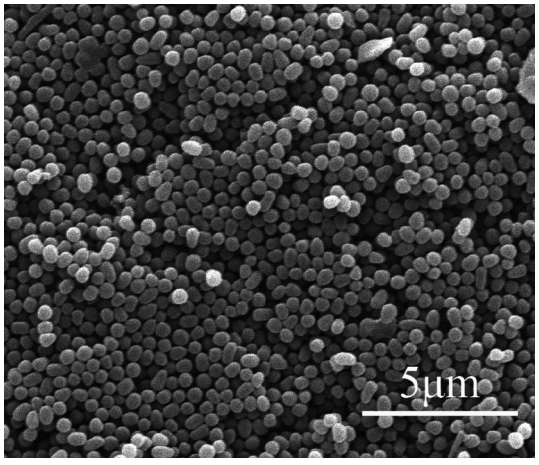

Fig. 1 Scanning electron micrograph of strain BL1

16S rRNA gene sequence analysis was performed during the logarithmic growth phase, and the sequence data were registered at GenBank (accession number HM136577). Through a Basic Local Alignment Search Tool (BLAST) search, homology analysis was performed to identify the known sequence in GenBank with the highest identity to the isolate. A phylogenetic tree (Fig. 2) was constructed using the MEGA 5.0 program, and the results determined that strain BL1 belongs to *Serratia marcescens*.

3.3 Degradation characteristics of strain BL1

3.3.1 Primary substrate

The environment for the domestication and enrichment of strain BL1 contained inorganic salts, methanol, and IBU. The purpose of adding methanol was to enhance solubility and provide a primary substrate. The BL1 colony obtained under such conditions did not proliferate and did not degrade IBU in an environment free of the primary substrate (Fig. 3). When methanol was added to the medium, the growth of BL1 returned to normal. During the logarithmic phase, the degradation of IBU increased significantly, while during the stationary phase, IBU degradation stabilized. At the end of cultivation, degradation of IBU reached 76.33%±4.51%, which indicated that IBU was not the only energy source for BL1, and that BL1 proliferation required the presence of a primary substrate. However, a study previously reported that IBU removal of 72.03% could be achieved in a moving bed biofilm reactor (MBBR) with a hydraulic retention time of 145.15 h and only IBU used as the substrate (Hoseinzadeh et al., 2016). However, the constitution of the MBBR was much more complex than that of our static test consisting of only the IBU solution and one type of bacterium. The co-existence of gas–liquid–bacterium colonies promotes the IBU degradation process, so the results of this study and the

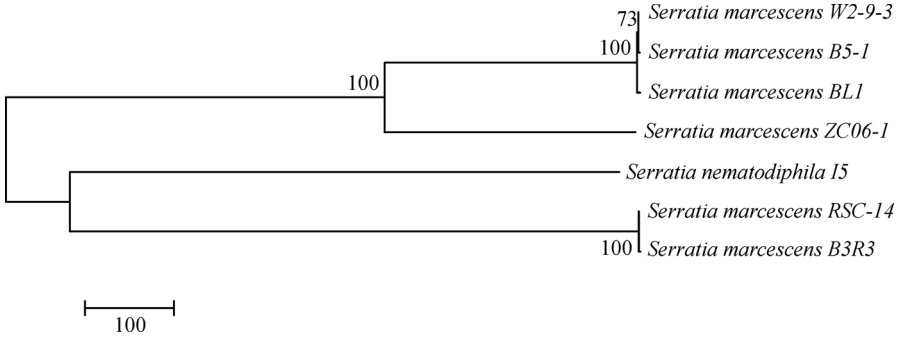

Fig. 2 Phylogenetic tree of strain BL1 based on 16S rDNA sequence (Numbers at the nodes indicate bootstrap percentages for 1000 replicates. The scale bars represent 100 substitutions/site)

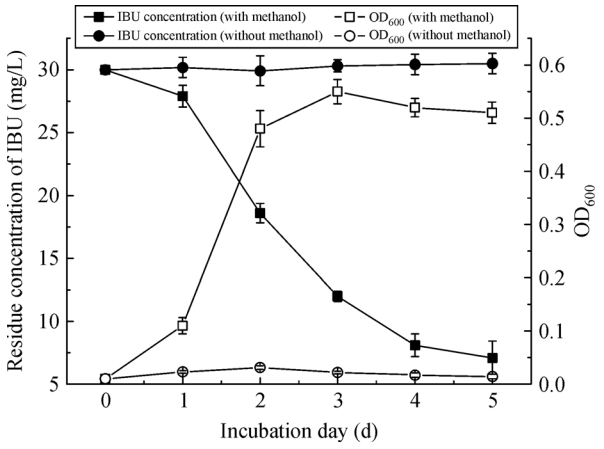

Fig. 3 Effect of methanol on the degradation process of IBU by strain BL1

MBBR study can not be directly compared. In short, in conjunction with the degradation strain, the primary substrate plays a key role in optimizing treatment of IBU.

Different primary substrates can affect the degradation efficiency of target contaminants (Guo et al., 2012; Trzcinski et al., 2016; Zhang et al., 2016), and significant differences were found in the degradation effect of IBU when yeast, peptone, and glucose were added to the culture medium (Fig. 4). The highest IBU degradation (93.47%± 2.37% on day 5) was achieved when yeast was used as the primary substrate, and the degradation of peptone and glucose was 84.13%±2.28% and 82.97%±5.50%, respectively. The process of IBU degradation under all primary substrate conditions followed first-order reaction kinetics ($P < 0.0002$), and the fitting equations are shown in Table 1. The degradation performance of BL1 was worse than that of electro-peroxone, which can achieve almost complete mineralization of IBU (Li et al., 2014; Zhang et al., 2016), but it was significantly better than those of the reported bacteria *Sphingomonas* spp. (50% within 10 d) (Li et al., 2016), *Sphingomonas* (30% within 2 d) (Murdoch and Hay, 2013), and *Bacillus thuringiensis* (46.56% within 20 d) (Marchlewicz et al., 2016).

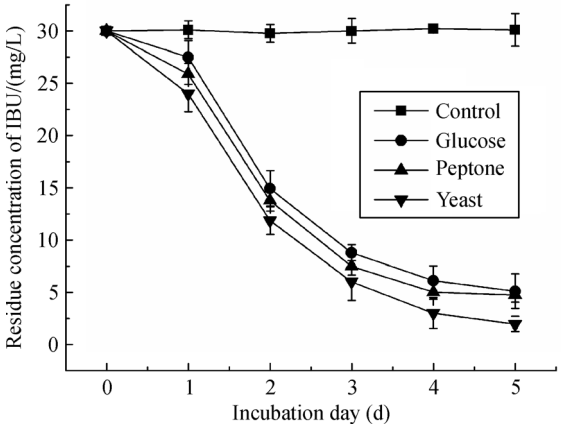

Fig. 4 Effect of nutrients on the degradation process of IBU by strain BL1

Monitoring of glucose metabolism revealed that the consumption rate of glucose was faster than that of IBU. A previous study indicated that co-metabolic substrates might induce key enzymes necessary for target pollutant degradation in addition to supporting growth (Liang et al., 2009). However, glucose utilization is quick and precedes target pollutant degradation (Pakshirajan et al., 2008). Thus, we speculated that BL1 preferentially consumed glucose for cell proliferation and synthesized the enzymes related to IBU degradation. Given the differences in IBU degradation caused by different primary substrates, we speculated that yeast powder enhanced the expression of proteins related to IBU degradation in this strain. When there was no primary substrate in the reaction system, the expression of these proteins was inhibited and IBU degradation was hindered.

3.3.2 Reaction temperature and pH

As shown in Fig. 5, the highest degradation of IBU (93.5±1.55%) was achieved at pH 7, and degradation of IBU was higher than 80% at pH 6–9, which indicated that a neutral pH environment was favorable for BL1. However, BL1 exhibited good adaptability for weakly acidic and weakly alkaline environments, and the physiologic and biochemical processes of BL1 were affected only at pH 5. At pH 5, IBU degradation was reduced to 67.3%±1.07%. These results indicate that BL1 adapts well to various pH environments. The highest degradation of IBU by BL1 (94.3%±1.59%) was achieved at 30°C, and degradation at 15°C was also higher than 50%, indicating that metabolism in BL1 proceeded normally at room temperature. In addition, the enzymatic activity was relatively high.

3.4 Application of strain BL1 to a simulated BAF system

Average COD_{Mn} removal by B1 and B2 was 28.74%± 7.77% and 29.86%±8.03%, respectively, and average

Table 1 Kinetic equations and parameters for the IBU removal under different primary substrate conditions

Primary substrate	Kinetic equation	K (d^{-1})	$t_{1/2}$ (d)	P-value	R^2
Glucose	$\ln C$ [a] $= -0.39t$ [b] $+ 3.50$	0.39	1.78	< 0.0002	0.97
Peptone	$\ln C = -0.42t + 3.46$	0.42	1.65	< 0.0002	0.95
Yeast	$\ln C = -0.58t + 3.57$	0.58	1.20	< 0.0002	0.99

Notes: a) C represented of concentration (mg/L) of IBU; b) t represented of cultivation time (d)

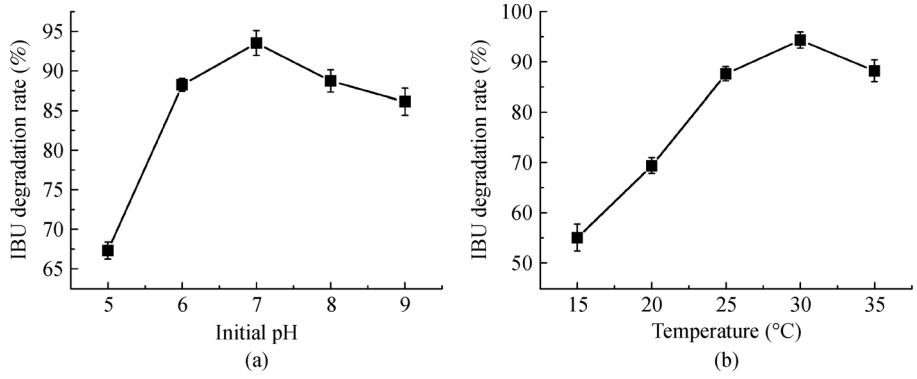

Fig. 5 Effect of initial pH and temperature on the degradation efficiency of IBU by strain BL1: (a) pH; (b) temperature

ammonia-nitrogen removal was 87.05%±5.85% and 89.08%±5.69%, respectively (Fig. 6(a)), indicating that there was no obvious advantage in the treatment of conventional pollutants by the BAF system with BL1. However, supplementation with strain BL1 did not negatively affect the removal of conventional pollutants. Because the isolated strain accounted for a small portion of the bacterial community in the BAF, the removal of COD_{Mn} and ammonia nitrogen could be attributed to the function of the indigenous bacterial community. The ability of the indigenous bacterial community to remove conventional pollutants was not influenced by strain BL1, which is advantageous for application of the strain in sewage treatment.

The removal of COD_{Mn} and ammonia-nitrogen gradually increased during the 15-d treatment, and IBU removal followed a similar trend: from 84.70%±1.91% at day 1 to 92.46%±1.76% at day 15 in B2. IBU removal was significantly different between B1 and B2 (Fig. 6(b)). Average IBU removal by B2 was 32.01%–44.04% higher than by B1. Recent studies reported an average IBU removal of about 50% in sewage by utilizing different sequencing batch reactors (Londoño and Peñuela, 2015; Abu Hasan et al., 2016), and this result was similar to the performance of B1. BL1 in the BAF performed as well as it did in the static tests, indicating that BL1 was able to promote IBU removal in a complex environment.

It is worth noting that IBU removal in the BAF was not significantly different from that in a shake flask. In the shake flask experiment, a single bacterium was inoculated in a nutrient-rich environment. In contrast, high concentrations of primary substrate were not added to the BAF, yet more than 90% of the treatment performance was still achieved. Speculative reasons for the similar results between the static and BAF tests are: 1) the metabolic contribution of other microorganisms in the biofilm, 2) the metabolic contribution of BL1, and 3) the ceramsite filter and the adsorption, filtration, and interception effects of the biofilm on the ceramsite surface. Further studies including culture-based assays, quantitative PCR (qPCR) and microarray are required to clarify the individual contributions of the above factors (Lin et al., 2016). In addition, studies have shown that high concentrations of IBU can inhibit biofilm formation (Londoño and Peñuela, 2015). However, COD_{Mn} and ammonia-nitrogen removal during the biofilm formation period was consistent with that of normal influent water, indicating that IBU had no toxic effects on the biofilms at 10 μg/L.

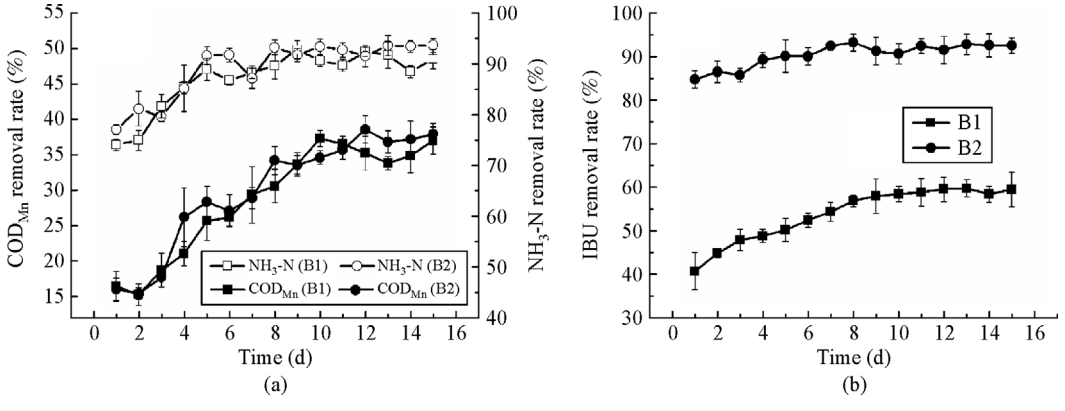

Fig. 6 Removal efficiencies of conventional pollution indicators and IBU by BAF: (a) COD_{Mn} and ammonia nitrogen; (b) IBU

4 Conclusions

An IBU-degrading strain was isolated from activated sludge of a sewage treatment plant. The strain was identified as *Serratia marcescens* BL1 by morphological, physiologic, and biochemical characterization as well as by genetic analysis. Optimal IBU degradation of BL1 was achieved at a culture temperature of 30°C and pH 7, with yeast powder as the primary substrate. The strain could not proliferate or metabolize IBU when IBU was the sole substrate. BL1 was applied to a BAF device and it formed a stable biofilm. Higher IBU removal was achieved in the BAF with the addition of BL1, but there was little difference in the removal of conventional pollutants.

Acknowledgements This work was funded by the National Natural Science Foundation of China (Grant Nos. 21767013 and 51741805) and the Natural Science Foundation of Jiangxi Province (No. 20151BAB213018).

References

Abu Hasan H, Sheikh Abdullah S R, Al-Attabi A W N, Nash D A H, Anuar N, Abd. Rahman N, Sulistiyaning Titah H (2016). Removal of ibuprofen, ketoprofen, COD and nitrogen compounds from pharmaceutical wastewater using aerobic suspension-sequencing batch reactor (ASSBR). Separation and Purification Technology, 157(1): 215–221

Ali I, Singh P, Aboul-Enein H Y, Sharma B (2009). Chiral analysis of ibuprofen residues in water and sediment. Analytical Letters, 42(12): 1747–1760

Baghapour M A, Shirdarreh M R, Faramarzian M (2015). Amoxicillin removal from aqueous solutions using submerged biological aerated filter. Desalination and Water Treatment, 54(3): 790–801

Chu H, Cao D, Dong B, Qiang Z (2010). Bio-diatomite dynamic membrane reactor for micro-polluted surface water treatment. Water Research, 44(5): 1573–1579

Cycoń M, Żmijowska A, Wójcik M, Piotrowska-Seget Z (2013). Biodegradation and bioremediation potential of diazinon-degrading Serratia marcescens to remove other organophosphorus pesticides from soils. Journal of Environmental Management, 117(1): 7–16

de Sousa D N, Mozeto A A, Carneiro R L, Fadini P S (2014). Electrical conductivity and emerging contaminant as markers of surface freshwater contamination by wastewater. Science of the Total Environment, 484(1): 19–26

Ferrando-Climent L, Collado N, Buttiglieri G, Gros M, Rodriguez-Roda I, Rodriguez-Mozaz S, Barceló D (2012). Comprehensive study of ibuprofen and its metabolites in activated sludge batch experiments and aquatic environment. Science of the Total Environment, 438(1): 404–413

Gao P, Wei X, Gu C, Wu X, Xue G, Shi W, Sun W (2015). Isolation and characterization of an erythromycin-degrading strain and application for bioaugmentation in a biological aerated filter. Water, Air, & Soil Pollution, 226(6): 190

Guo H, Yao J, Chen H, Wang J, Masakorala K, Jin Y, Richnow H H, Blake R E (2012). Substrate interactions during biodegradation of benzene/alkylbenzene mixtures by *Rhodococcus* sp. ustb-1. International Biodeterioration & Biodegradation, 75(1): 124–130

Halling-Sørensen B, Nors Nielsen S, Lanzky P F, Ingerslev F, Holten Lützhøft H C, Jørgensen S E (1998). Occurrence, fate and effects of pharmaceutical substances in the environment–a review. Chemosphere, 36(2): 357–393

Han S, Choi K, Kim J, Ji K, Kim S, Ahn B, Yun J, Choi K, Khim J S, Zhang X, Giesy J P (2010). Endocrine disruption and consequences of chronic exposure to ibuprofen in Japanese medaka (*Oryzias latipes*) and freshwater cladocerans *Daphnia magna* and *Moina macrocopa*. Aquatic Toxicology (Amsterdam, Netherlands), 98(3): 256–264

He S, Wang J, Ye L, Zhang Y, Yu J (2014). Removal of diclofenac from surface water by electron beam irradiation combined with a biological aerated filter. Radiation Physics and Chemistry, 105(1): 104–108

Hoseinzadeh E, Rezaee A, Hossini H (2016). Biological nitrogen removal in moving bed biofilm reactor using ibuprofen as carbon source. Water, Air, and Soil Pollution, 227(2): 46

Li X, Wang Y, Yuan S, Li Z, Wang B, Huang J, Deng S, Yu G (2014). Degradation of the anti-inflammatory drug ibuprofen by electro-peroxone process. Water Research, 63(1): 81–93

Li Y, Wu B, Zhu G, Liu Y, Ng W J, Appan A, Tan S K (2016). High-throughput pyrosequencing analysis of bacteria relevant to cometabolic and metabolic degradation of ibuprofen in horizontal subsurface flow constructed wetlands. Science of the Total Environment, 562(1): 604–613

Liang Y, Zeng F, Qiu G, Lu X, Liu X, Gao H (2009). Co-metabolic degradation of dimethoate by *Raoultella* sp. X1. Biodegradation, 20 (3): 363–373

Lin Y, Li D, Zeng S, He M (2016). Changes of microbial composition during wastewater reclamation and distribution systems revealed by high-throughput sequencing analyses. Frontiers of Environmental Science & Engineering, 10(3): 539–547

Lindholm-Lehto P C, Ahkola H S J, Knuutinen J S, Herve S H (2016). Widespread occurrence and seasonal variation of pharmaceuticals in surface waters and municipal wastewater treatment plants in central Finland. Environmental Science and Pollution Research International, 23(8): 7985–7997

Londoño Y A, Peñuela G A (2015). Biological removal of different concentrations of ibuprofen and methylparaben in a sequencing batch reactor (SBR). Water, Air, and Soil Pollution, 226(12): 393

Marchlewicz A, Domaradzka D, Guzik U, Wojcieszyńska D (2016). Bacillus thuringiensis B1(2015b) is a gram-positive bacteria able to degrade naproxen and ibuprofen. Water, Air & Soil Pollution, 227(6): 197

Marchlewicz A, Guzik U, Hupert-Kocurek K, Nowak A, Wilczyńska S, Wojcieszyńska D (2017). Toxicity and biodegradation of ibuprofen by *Bacillus thuringiensis* B1(2015b). Environmental Science and Pollution Research International, 24(8): 7572–7584

Ministry of Environmental Protection of the People's Republic of China (1989). Water quality-Determination of permanganate index. Available online at http://kjs.mep.gov.cn/hjbhbz/bzwb/jcffbz/199007/t19900701_67130.shtml (accessed July 19, 2018) (in Chinese)

Ministry of Environmental Protection of the People's Republic of China (2009). Water quality-Determination of ammonia nitrogen-Nessler's reagent spectrophotometry. Available online at http://kjs.mep.gov.cn/hjbhbz/bzwb/jcffbz/201001/t20100112_184155.shtml (accessed July 19, 2018) (in Chinese)

Murdoch R W, Hay A G (2013). Genetic and chemical characterization of ibuprofen degradation by *Sphingomonas* Ibu-2. Microbiology, 159 (Pt_3): 621–632

Paíga P, Santos L H M L M, Amorim C G, Araújo A N, Montenegro M C, Pena A, Delerue-Matos C (2013). Pilot monitoring study of ibuprofen in surface waters of north of Portugal. Environmental Science and Pollution Research International, 20(4): 2410–2420

Pakshirajan K, Chugh D, Saravanan P (2008). Feasibility of m-cresol degradation using an indigenous mixed microbial culture with glucose as co-substrate. Clean Technologies and Environmental Policy, 10(3): 303–308

Parolini M, Binelli A, Provini A (2011). Chronic effects induced by ibuprofen on the freshwater bivalve *Dreissena polymorpha*. Ecotoxicology and Environmental Safety, 74(6): 1586–1594

Phan H V, Hai F I, Mcdonald J A, Khan S J, Zhang R, Price W E, Broeckmann A, Nghiem L D (2015). Nutrient and trace organic contaminant removal from wastewater of a resort town: comparison between a pilot and a full scale membrane bioreactor. International Biodeterioration & Biodegradation, 102(1): 40–48

Pietrini F, Di Baccio D, Aceña J, Pérez S, Barceló D, Zacchini M (2015). Ibuprofen exposure in *Lemna gibba* L.: Evaluation of growth and phytotoxic indicators, detection of ibuprofen and identification of its metabolites in plant and in the medium. Journal of Hazardous Materials, 300(1): 189–193

Qim Y, Li D, Yang H (2007). Population diversity and community structure of bacteria on biofilms in a potable water pretreating bioreactor. Chinese Journal of Applied and Environmental Biology, 13(1): 104–107

Shen P, Chen X (2007).Microbiology experiment. In: Shen P, Chen X, eds. Physiological and Biochemical Experiment for Microorganism Identification. 4th ed. Beijing: Higher Education Press, 111–127(in Chinese)

Tran N H, Li J, Hu J, Ong S L (2014a). Occurrence and suitability of pharmaceuticals and personal care products as molecular markers for raw wastewater contamination in surface water and groundwater. Environmental Science and Pollution Research International, 21(6): 4727–4740

Tran N H, Urase T, Ta T T (2014b). A preliminary study on the occurrence of pharmaceutically active compounds in hospital wastewater and surface water in Hanoi, Vietnam. Clean- Soil Air Water, 42(1): 267–275

Trzcinski A P, Ganda L, Kunacheva C, Zhang D Q, Lin L L, Tao G, Lee Y, Ng W J (2016). Characterization and biodegradability of sludge from a high rate A-stage contact tank and B-stage membrane bioreactor of a pilot-scale AB system treating municipal wastewaters. Water Science &Technology, 74(7): 1716–1725

Wang K, Li W, Gong X, Li X, Liu W, He C, Wang Z, Minh Q N, Chen C, Wang J (2014). Biological pretreatment of tannery wastewater using a full-scale hydrolysis acidification system. International Biodeterioration & Biodegradation, 95(Part A): 41–45

Zhang Y, Lv T, Carvalho P N, Arias C A, Chen Z, Brix H (2016). Removal of the pharmaceuticals ibuprofen and iohexol by four wetland plant species in hydroponic culture: plant uptake and microbial degradation. Environmental Science and Pollution Research International, 23(3): 2890–2898

Zhao W, Guo Y, Lu S, Yan P, Sui Q (2016). Recent advances in pharmaceuticals and personal care products in the surface water and sediments in China. Frontiers of Environmental Science & Engineering, 10(6): 2