Water Quality Research Journal

Water Quality Research Journal Vol 58 No 2, 75 doi: 10.2166/wqrj.2023.003

Potential of *Pseudomonas yamanorum* for the valorization of municipal biosolids

Jordan Lussier, Linson Lonappan and Hubert Cabana *

Environmental Engineering Laboratory, Department of Civil and Building Engineering, Université de Sherbrooke Water Research Group, Sherbrooke, QC J1K 2R1, Canada

*Corresponding author. E-mail: hubert.cabana@usherbrooke.ca

HC, 0000-0003-3417-6816

ABSTRACT

It is generally accepted that some trace organic contaminants (TrOCs) pass through the wastewater treatment process without being properly treated and find their way into waterbodies. These molecules can also be concentrated within the biosolids (BS) through adsorption. The presence of TrOCs in BS, which are then commonly used as soil amendments in agriculture, may affect plant growth and viability. The potential risks posed by TrOCs are usually ignored because they are present in low concentrations and mostly have relatively short half-lives. However, the continuous addition of these substances in water sources and on farmlands makes them pseudo-persistent. To reduce the concentrations of selected TrOCs from these BS, *Pseudomonas yamanorum* LBUM636 (PY) was tested with and without a commercial bacterial blend of *Bacillus* spp. (BC). About 60% removal of atrazine was achieved using PY-amended BS. Bioslurries inoculated with PY had relatively high laccase activity at about 2,200 U/L. Laccase activity was seven times higher in samples where BC was also present, which suggests a synergistic effect between BC and PY. Concentrations of phenazine-1-carboxylic acid, an antibiotic with a biopesticide effect, were also relatively important in PY-inoculated bioslurries.

Key words: atrazine, biosolids, laccase, phenazine-1-carboxylic acid, *Pseudomonas yamanorum*, trace organic contaminants

HIGHLIGHTS

- *Pseudomonas yamanorum* was successfully grown on municipal biosolids (BS) with/without the addition of a commercial blend of *Bacillus* spp.
- *P. yamanorum* induced the reduction of atrazine from BS.
- Phenazine-1-carboxylic acid was produced in *P. yamanorum* inoculated BS.

INTRODUCTION

Effective removal of trace organic contaminants (TrOCs), such as pesticides and pharmaceuticals, from wastewater, is becoming an important issue to address since the long-term effects of TrOCs can be harmful to aquatic environments and human health (Daughton 2010). Although hydrophobic TrOCs are more likely to be removed from wastewaters by sorption to sludge, the removal efficiency can still be limited from one wastewater treatment plant (WWTP) to another depending on the type of treatment process used and various conditions such as temperature, retention time (solid and hydraulic) and even the dilution of wastewater caused by rainwater (Vieno *et al.* 2007). To achieve an effective removal of many TrOCs, costly and complex physico-chemical processes capable of processing the daily peak flow rate would need to be developed and implemented (World Health Organization 2012).

Several TrOCs removed during the wastewater treatment process get concentrated in sludge, which may then be valorized in agriculture as a soil amendment. Biosolids (BS) typically contain phosphorus, nitrogen, potash, proteins, grease and fat, cellulose, various trace metallic elements and TrOCs (Vaithyanathan *et al.* 2020). Many of these promote bacterial growth and can benefit plant growth. If the undesirable trace metallic elements and TrOCs can be removed effectively, the BS can be safely used as a nutrient source for plants.

According to Vieno *et al.* (2007) and Spahr *et al.* (2020), various contaminants can be partially transformed or removed through biologically supported and abiotic mechanisms. The extent of the transformation or removal depends on the

sludge stabilization process used and it is generally reported that not all adsorbed TrOCs can be transformed by a given process (Vieno *et al.* 2007; Spahr *et al.* 2020). Even if they could be, there are many transformation products that may be as harmful as or more harmful than the parent TrOC molecule (Yang *et al.* 2016; Spahr *et al.* 2020). These transformation products will still get released into the environment and could eventually reach our food chain through desorption from sludge on croplands.

Therefore, an economical and simple solution is required to reduce the TrOC load in municipal BS. Bioaugmentation (El Fantroussi & Agathos 2005), which consists of isolating and using the most effective strains to transform TrOCs from a given environmental matrix, is one of the promising options (Wattiau *et al.* 2001; Drouin *et al.* 2008). Various microorganisms could have the potential to transform a variety of compounds found in BS through various mechanisms (Olicón-Hernández *et al.* 2017). To survive and thrive in municipal sludge, it is essential for the selected microorganism to be resistant to endogenous microorganisms as well as various contaminants that may be present in municipal BS such as fertilizers, pesticides, pharmaceuticals and heavy metals.

Pseudomonas genus has demonstrated a good tolerance level to some heavy metals, such as lead, in addition to its ability to remove lead (Vélez *et al.* 2021). *Pseudomonas* spp. uses various mechanisms including biosorption, bioaccumulation and the production of exopolysaccharides, to survive in the presence of heavy metals such as lead (Vélez *et al.* 2021).

Atrazine can be transformed by *Pseudomonas* spp. through an oxidative pathway and a hydrolytic pathway (Belal *et al.* 2013; Tonelli Fernandes *et al.* 2018). Transformation via the oxidative pathway is done under aerobic conditions via dealkylation, dechlorination, deamination and ring cleavage (Belal *et al.* 2013; Tonelli Fernandes *et al.* 2018). Transformation via the hydrolytic pathway is done via dehalogenation, *N*-dealkylation, deamination and ring cleavage (Belal *et al.* 2013). In both cases, it leads to the formation of cyanuric acid which can then be further mineralized to form CO_2 and NH_3 (Belal *et al.* 2013; Tonelli Fernandes *et al.* 2018). The toxic effects of atrazine on humans, plants, animals and various microorganisms have been extensively studied and well-established. Generally, atrazine causes oxidative stress in some non-target plants and microbes, whereas, in humans, it disrupts the hypothalamic control of pituitary-ovarian function (Singh *et al.* 2018). However, previous studies reported that the metabolites formed through biodegradation showed fewer toxic effects than the parent compound, atrazine (Kross *et al.* 1992; Kolekar *et al.* 2014).

The aim of this article is to present the potential of using *P. yamanorum* LBUM636 (PY) (formerly known as *P. fluorescens* LBUM636) for BS decontamination and valorization, whether it is used alone or with a blend of commercially available microorganisms to improve BS treatments. To demonstrate this, the removal of atrazine from BS accumulated in sewage treatment plants and the production of phenazine-1-carboxylic acid (PCA), an antibiotic, produced by PY-inoculated BS, are presented and discussed in this paper. As a basis for comparison, a commercial product (BactoCharge (BC)), used for sludge decontamination, has been selected to compare PY efficiency. This product contains mostly *Bacilli* strains that are known to be efficient for wastewater treatment (Wattiau *et al.* 2001; Drouin *et al.* 2008; Vaithyanathan *et al.* 2021). Amylase, lipase and protease are also in the commercial product to increase the initial rate of treatment. In addition, a preliminary evaluation is carried out of the compatibility of and synergy between PY and BC. Since PCA can act as a natural pesticide, quantifying its production is also of great interest and may give added value to treated BS used in selected applications in agriculture.

MATERIALS AND METHODS

Chemicals

All reagents used were of analytical grade or better. 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), veratryl alcohol, para-nitrophenyl phosphate, casein, para-nitrophenyl palmitate, soluble starch and pesticide standard, supplied in powder form (purity $> 95\%$), were purchased from Sigma-Aldrich (Saint-Louis, MO, USA). Water was purified and deionized by a Milli-Q purification system (minimum resistivity 18 MΩ cm; Millipore, Billerica, MA, USA).

P. yamanorum LBUM636 was provided by NUVAC Eco-Sciences Inc. Bacteria from BactoCharge (BC), a commercially available dry powder product (NUVAC Eco-Sciences Inc, Valcourt, QC, Canada), composed of a *Bacilli* blend was used as a parallel treatment for comparison with PY.

BS sampling and characterization

BS and wastewater effluent (WWE) were obtained from a local municipal WWTP in the province of Quebec, Canada. The BS collected were kept frozen (−18 °C) and the effluent was stored at 4 °C prior to use. A 50% (m/v) BS slurry was prepared by adding 50 g of dry BS in 100 mL of effluent in a 500 mL Erlenmeyer flask.

Volumetric parameters such as total solids (TS), total dissolved solids (TDS) and total suspended solids (TSS) in the bioslurry were measured according to standard methods (American Public Health Association 2023). After measuring the volumetric parameters of the bioslurry, the samples were centrifuged at 4,500 rpm (1.8× *g*) for 10 min to obtain pellet and supernatant. The supernatant was filtered through a 0.45 μm membrane. The activities of hydrolytic and oxidoreductase enzymes, such as laccase, lignin peroxidase, aryl alcohol oxidase, protease, phosphatase, lipase and amylase were assayed in the supernatant of the bioslurry according to previously established studies (Folin 1929; Tabatabai & Bremner 1969; Kumar & Rapheal 2011; Yu *et al.* 2013; Touahar *et al.* 2014; Rathankumar *et al.* 2019).

Inoculum preparation

A one-liter PY preculture with Tryptic Soy Broth was initially prepared in a 2 L Erlenmeyer flask placed on a rotating plate for 24 h (Oberhofer 1979). This culture was then transferred into a 7 L bioreactor, containing 5 L of fresh culture broth, and left for 48 h with aeration of 30 mmol/L/h and agitation at 150 rpm. The resultant culture was centrifuged at 4,500 rpm (1.8× *g*) for 15 min, the supernatant was then discarded, and the PY inoculum was obtained from the pellet. The BC was inoculated directly in the BS at the beginning of the experiment.

Bioaugmentation by PY and BC using aerobic digestion

To obtain a greater range of results, the strain's performance was evaluated over a 90-day period with samples being taken every 30 days (on Days 0, 30, 60 and 90).

Bioslurry samples of 100 mL (TSS at 43.85 g/L and TDS at 1.65 g/L) were inoculated with 10% (w/v) of a concentrated PY culture in a 500 mL Erlenmeyer flask. A similar procedure was applied with BC. A third sample group, where both PY and BC were co-inoculated at a concentration of 10% (w/v), was prepared. To ensure that BC did not prevent the growth of PY, BC was added to the samples 3 days after PY. A negative control sample group was also prepared in the same way except these samples were not inoculated. All the flasks were incubated at 27 °C with continuous orbital agitation at 150 rpm for 90 days. Each sample was prepared in duplicate and samples from each treatment were analyzed twice.

After measuring the volumetric parameters in the bioslurries, the samples were centrifuged, and various parameters of the supernatant were characterized as mentioned in the BS sampling and characterization section. The atrazine concentration in each bioslurry phase was measured at 30-day intervals. Atrazine removal efficiency from BS was calculated as the difference between the initial and final concentration in both the liquid and solid phases (Equation (1)).

$$\mathrm{TrOCs}_{\mathrm{removal}} = \frac{\mathrm{TrOCs}_{\mathrm{Initial}}\ (\mathrm{BS}) - \mathrm{TrOCs}_{\mathrm{Final}}\ (\mathrm{Solid\ phase}_{\mathrm{after\ 90\ days}})}{\mathrm{TrOCs}_{\mathrm{Initial}}(\mathrm{BS})} \tag{1}$$

To compare the samples with the control, a statistical variance analysis (ANOVA) was performed using a Holm–Sidak test (Sigma plot, version 11, Systat Software Inc), wherever necessary. The level of significance is expressed as the *P*-value < 0.05.

Atrazine and PCA extraction and analysis

Analysis of the presence of atrazine and PCA was done through UPLC-MS/MS according to a previously developed method (Haroune *et al.* 2015; Rathankumar *et al.* 2020). Atrazine and PCA were extracted from the bioslurry using a QuEChERS method (Zhang *et al.* 2019).

Quantification of the atrazine and PCA was carried out with an Ultra Performance Liquid Chromatography (UPLC), Acquity UPLC system, with a reverse phase C18 HSS T3 1.8 μm column, 2.1 × 50 mm from Waters Corporation (Waters Corporation, Milford, MA, USA). This device is coupled to a tandem quadrupole mass spectrometer, Acquity UPLC XEVO TQ detector. Quantification of the molecules was performed by electro-nebulization in positive mode MS/MS-ESI (+). The mobile phases consisted of water + 0.1% formic acid (H_2O + 0.1% AF) and methanol + 0.1% formic acid (MeOH + 0.1% AF). The following parameters were used: cone voltage 20 V, capillary voltage 2.5 kV, temperature 150 °C, desolvation temperature 450 °C, desolvation gas (N_2) at 800 L/h, cone gas (N_2) at 50 L/h and collision gas (Ar) at 0.38 mL/h.

RESULTS

Atrazine removal

Figure 1 shows that after 90 days of inoculation, the atrazine concentration was relatively similar in the solid fraction for all inoculated samples compared to the initial concentration. BC + PY-inoculated samples had a slightly greater overall removal

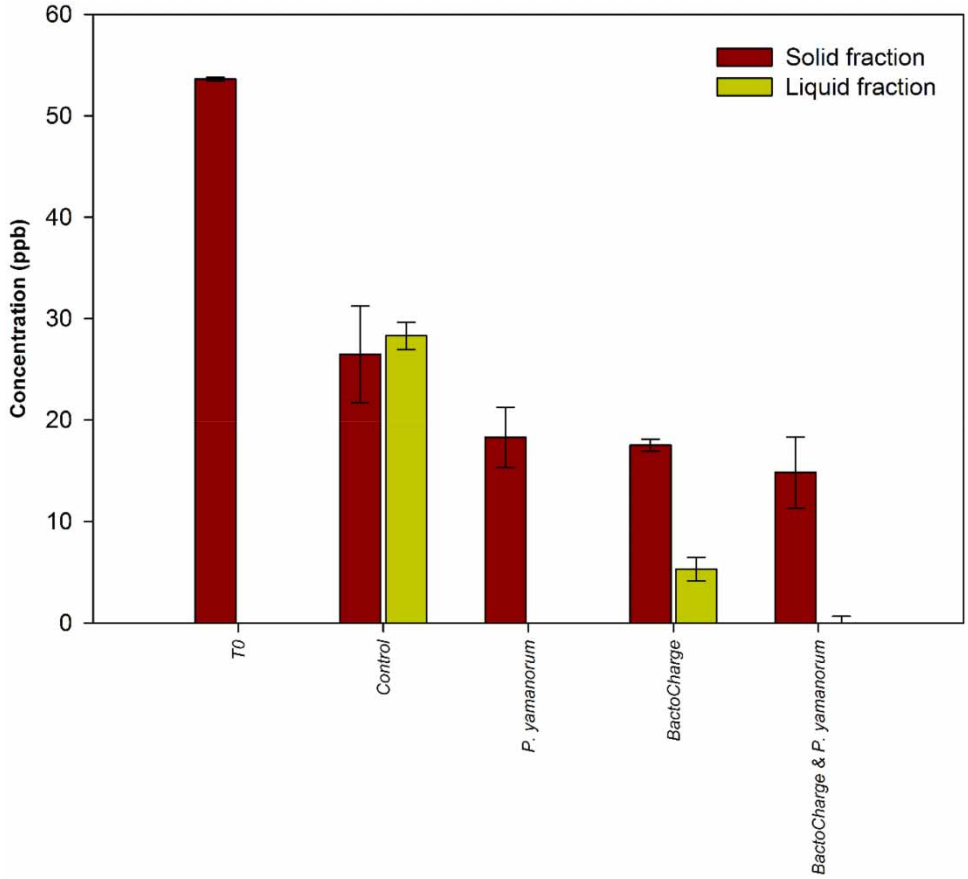

Figure 1 | Concentrations of atrazine in the solid and liquid fractions for all samples at T_0 and T_{90} days after inoculation.

of atrazine at 72% from the solid and liquid fractions, followed closely by PY-treated samples with a 66% removal of atrazine. The PY-inoculated samples had no detectable traces of atrazine in the liquid phase and a residual concentration in the solid phase similar to the BC samples. However, in the BC samples, traces of atrazine remained in the liquid fraction, which could indicate a reduced atrazine removal efficiency rate. The combination of PY with BC appears to have resulted in an atrazine removal rate similar to the PY treatment removal rate. At the very least, it can be stated that the combination of PY and BC did not reduce the atrazine removal effectiveness. The control showed a partial transfer of atrazine from the solid phase to the liquid phase but no quantifiable removal of atrazine. Results from Figure 1 also suggest that PY can effectively remove atrazine on its own. In comparison with the control, no statistically significant difference was observed for atrazine removal in solid fraction (P-value $= 0.089$); whereas a significant difference in removal was observed in liquid fraction (P-value < 0.001).

pH evolution

As can be seen in Figure 2, the pH of all the inoculated samples increased, while the pH of the control samples steadily decreased over time from 6 to 3. In comparison with the control samples, a statistically significant difference in pH was observed for all of the other samples for Day 30, Day 60 and Day 90 (P-value < 0.001). The pH of the PY samples declined between Day 60 and Day 90 from pH 7 to 5, which could indicate that there was not enough PY in the inoculated sample to maintain its optimal pH for more than 60 days after inoculation. As expected from the commercially available blend, the pH of the BC samples increased from 6 to 8 during the first 30 days and then gradually stabilized at a pH of approximately 7 for the remainder of the experiment. It can also be observed that after an initial pH of 6 at T_0, the pH of the PY + BC samples increased even more, reaching a pH as high as 10 and remaining at 8 or above during the entire experiment.

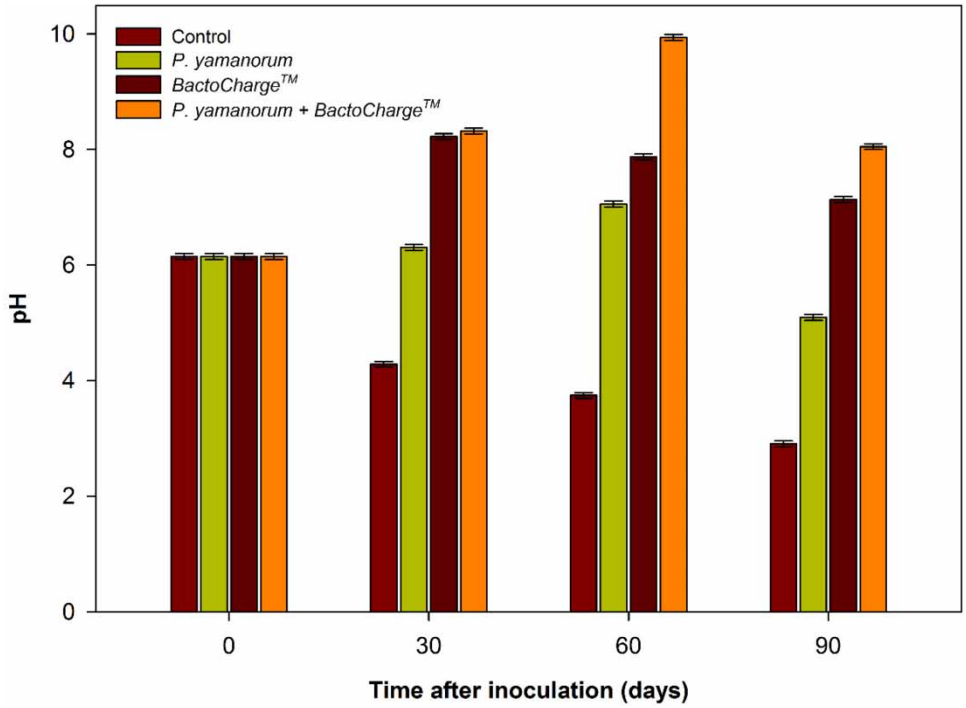

Figure 2 | pH evolution at 30-day intervals over 90 days after inoculation.

Enzymatic activity

Figure 3 shows that enzymatic activities for laccase, lipase, phosphatase and amylase were higher in PY-inoculated samples than in the control group, which indicates that samples containing PY were enzymatically more active than the control group for those enzymes. The same can be observed in the BC samples for lipase, phosphatase and amylase. As for the combination PY + BC, it promoted a significantly higher laccase activity (seven times greater than the PY-treated sample). As presented in Figure 3, the enzymatic activity for laccase on Day 30 for the combination PY + BC was significantly ($P = 0.001$) higher than the control samples or any other enzymatic activity measured during the entire experiment. However, no significant difference in laccase activity was observed for other samples in comparison with the control samples on Days 60 and 90, respectively (P-value = 0.134 and P-value = 0.053). Since laccase was found in PY and PY + BC samples but not in the control or BC samples, it can be concluded that PY may be mainly responsible for the production of laccase. However, the laccase concentration is about seven times higher in PY + BC than in PY samples, which seems to indicate a synergistic effect between BC and PY leading to increased laccase production. All the other enzymatic activities, however, are lower in PY + BC than in PY or BC, which suggests that the production of these enzymes is not favored when PY is combined with BC.

In the case of lipase, a significant (P-value < 0.001) increase in production was observed for all samples on Days 60 and 90 compared to the control samples. On the starting day, phosphatase production was constant in all samples and a significant increase in the production (P-value < 0.001) of phosphatase was observed for the combination PY + BC on Day 30. However, the Day 60 results did not reveal a noticeable increase in phosphatase activity between the control and other samples, which was further confirmed using ANOVA analysis (P-value = 0.463). An exceptional increase in amylase activity was observed for PY on Day 30 (P-value < 0.001). While comparing with the control samples, an increase in amylase activity was observed for all samples on Day 60; though activity in PY slightly decreased from Day 30. As can be seen in Figure 3, aryl alcohol oxidase production was negligible in inoculated samples with only very small amounts present in PY (about 5 units/L) and BC (about 20 units/L) on Day 90, which was lower than what was observed for the control group (about 67 units/L) on Day 60. As for lignin peroxidase, relatively high activity was measured on Day 60 in all the samples including the control samples and no statistically significant difference (P-value = 0.171) was observed between the control samples and the rest of the samples. Since there is no significant difference in activity between the control group and all the other samples, lignin peroxidase activity is not linked to the presence of PY or BC. Results were negligible for all samples (Figure 3) on Day 90.

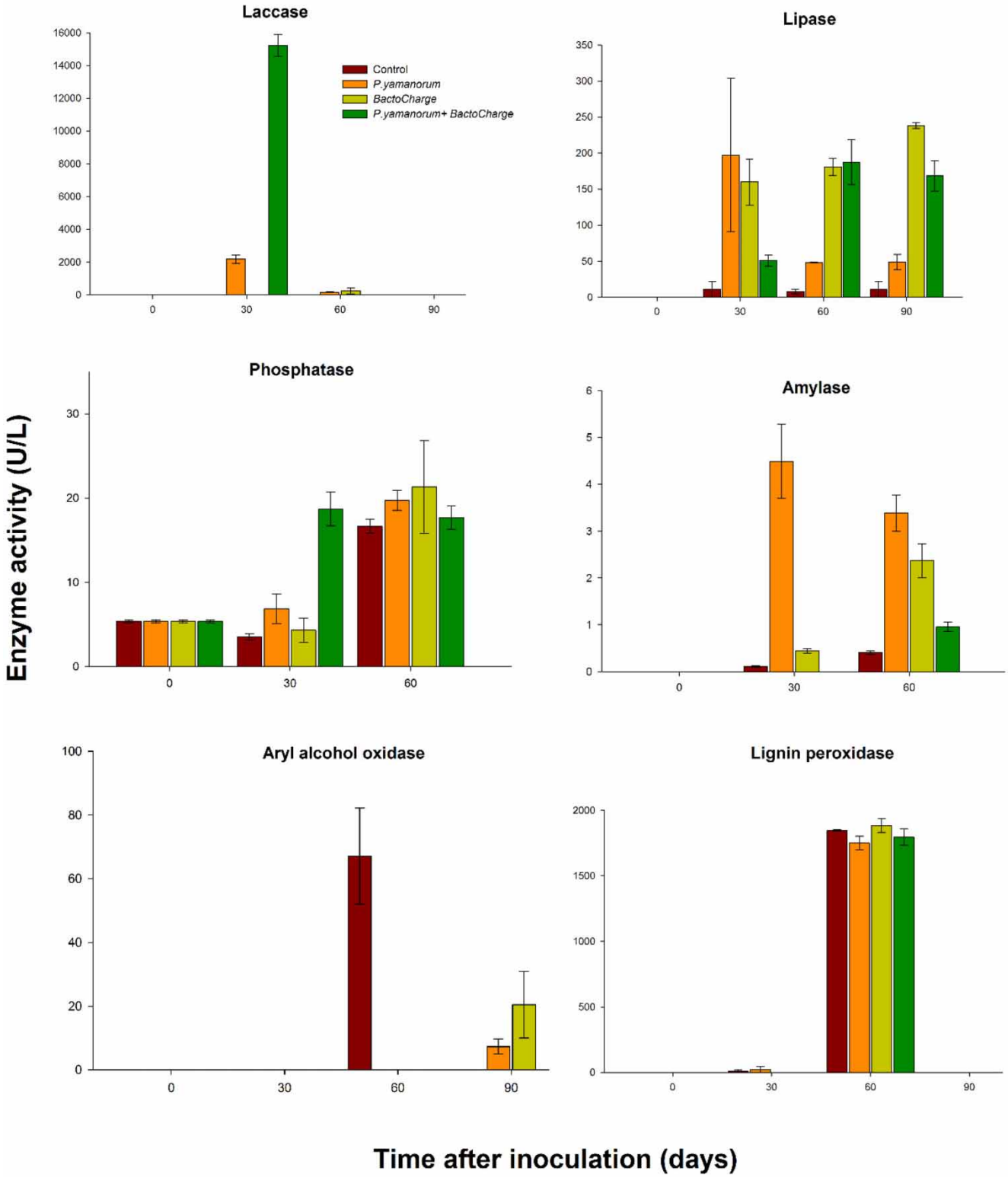

Figure 3 | Enzyme activities at 30-day intervals over 90 days after inoculation.

Phenazine-1-carboxylic acid production

Figure 4 shows that PCA was mostly found in the liquid phase of the *P. fluorescens* samples. The highest concentrations were found in the PY samples (about 300 μg/L) and in the samples inoculated with BC and PY (about 60 μg/L). However, when PY was combined with BC, the production of PCA was about five times lower than in samples with PY alone.

DISCUSSION

According to the literature, PY can produce hydrolytic and ligninolytic enzymes such as laccase and lignin peroxidase involved in the transformation mechanisms of TrOCs in BS (Belal *et al.* 2013; Janusz *et al.* 2017; Tonelli Fernandes *et al.* 2018). Ligninolytic enzymes are of particular interest for their ability to efficiently transform many TrOCs (Yang *et al.* 2017; Asif *et al.* 2018). Therefore, it may be possible to significantly reduce the concentration of TrOCs with an adequate consortium of microorganisms.

The results presented in Figures 1 and 3 for PY suggest that laccase is effective in removing atrazine. This is supported by other recent studies where the elimination of atrazine by laccase in BS was evaluated (Vaithyanathan *et al.* 2022). However, the results in Figure 1 do not show a significantly higher atrazine removal efficiency for PY + BC than for PY even though the laccase activity is seven times greater for PY + BC than it is for PY. This might be explained by the reduced presence in the PY + BC treated samples of other enzymes capable of atrazine removal such as lignin modifying enzymes and cytochrome P450 (Belal *et al.* 2013; Haroune *et al.* 2017; Tonelli Fernandes *et al.* 2018). The BC samples showed similar results to the PY samples for atrazine removal, even though no laccase was present in the BC cultures. Furthermore, it has been observed that many strains of *Bacillus*, including *B. subtilis*, are capable of producing laccase and can be effectively used for

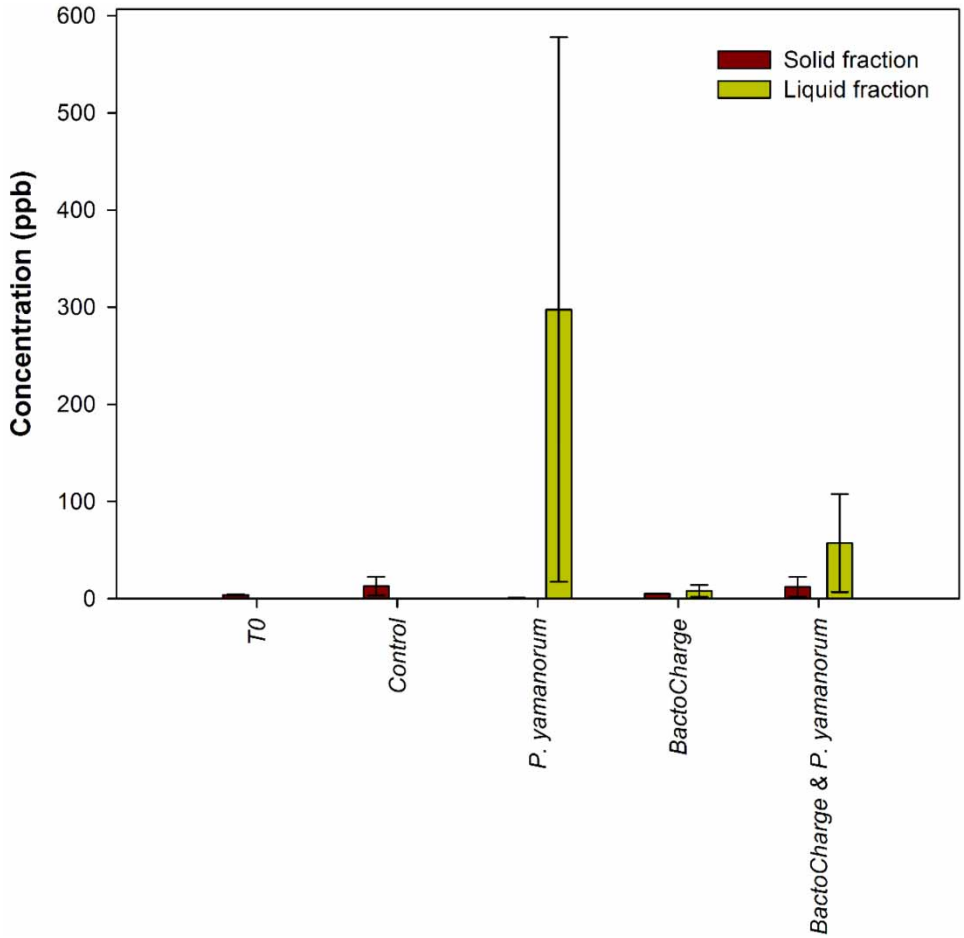

Figure 4 | Concentrations of phenazine-1-carboxylic acid (PCA) in the solid and liquid fraction samples at T_0 and T_{90} days after inoculation.

bioremediation purposes (Sheikhi *et al.* 2012). Under the culture conditions used in this study, the fact that BC did not produce laccase may be explained by an environmental stress that may have promoted the production of other enzymes and intracellular and extracellular mechanisms such as biosorption, dehalogenation, dealkylation, dechlorination, deamination and ring cleavage that can favor the removal of atrazine (Belal *et al.* 2013; Tonelli Fernandes *et al.* 2018; Vaithyanathan *et al.* 2022). It is also possible that the peak of laccase production in BC was reached between sampling points, which would make it appear as if there was very little laccase activity. Individually, many of the *Bacillus* spp. and *Pseudomonas* spp. have been confirmed to be very efficient at removing atrazine (Wang *et al.* 2014; Zhu *et al.* 2019; Singh *et al.* 2020). It can be observed from Figure 3 that enzyme activity was lower for lipase, phosphatase, amylase and aryl alcohol oxidase and unchanged for lignin peroxidase when PY and BC were combined. In other studies, it was observed that the presence of PY can promote laccase activity in other microorganisms (Johansen & Olsson 2005). This could mean that PY is not directly responsible for laccase production but renders the BS more favorable for other beneficial microorganisms and promotes mechanisms which require a pH near 7.

While laccase may be a key enzyme in atrazine removal and potentially many other TrOCs, the results did not demonstrate a significantly greater overall removal rate between the PY + BC samples with the highest concentration and the PY or BC samples with much lower laccase activity. Since the activity of other monitored enzymes was not significantly different for the various inoculated samples, it might be deduced that other enzymes and mechanisms induced by the presence of PY and BC must also contribute to the atrazine removal potential such as dehalogenation, dealkylation, dechlorination, deamination and ring cleavage (Belal *et al.* 2013; Tonelli Fernandes *et al.* 2018). This would explain the relatively similar atrazine removal rate in all the inoculated samples. However, results do show a tendency for a slightly higher atrazine removal rate in the samples inoculated with PY and BC than in samples containing only one of them. Therefore, it can be speculated that the combination of PY with BC might yield a slightly higher atrazine removal rate and possible synergy between the microorganisms in BC with PY. Synergistic relationships have been observed in other studies between PY and *B. subtilis* as well as with *B. amyloliquefaciens* for the well-being of plants and prevention of charcoal rot disease (Gamez *et al.* 2019; Ahmid & Ismail 2020). Since various microorganisms have multiple mechanisms resulting in various pathways that can lead to the transformation of a given TrOC such as atrazine, it is highly probable that PY and BC mechanisms are favoring each other's atrazine transformation pathways, resulting in a more efficient and complete transformation of atrazine than these microorganisms can achieve individually with their own atrazine transformation pathways.

The effectiveness of laccase to remove common TrOCs is also presented in another study in which laccase has a removal efficiency of at least 50% for 29 out of the 30 TrOCs selected, 13 of which had a $> 90\%$ removal efficiency (Asif *et al.* 2018). Various studies clearly demonstrate how highly effective laccase is in removing a variety of TrOCs from BS (Asif *et al.* 2018; Vaithyanathan *et al.* 2022). This confirms that laccase-producing microorganisms, such as BC and PY, are useful for the degradation of atrazine through laccase production (Vaithyanathan *et al.* 2022).

A clear distinction can be seen between the pH evolution of the control samples where the pH decreased steadily from 6 to 3 during the entire experiment and that of all the inoculated samples where the pH increased to 7–8. While the pH decreased in the PY samples during the last 30 days, the final pH was still significantly higher than it was in the control samples, which demonstrates that the presence of PY has a beneficial impact in regulating pH levels within the optimal range (pH 6–9.5) required by law in several jurisdictions for effluents emerging from WWTP (Regulation respecting municipal wastewater treatment works, 2020). Also, the concomitant presence of BC and PY provoked an even greater increase than either one on their own, which suggests that a synergistic effect occurs for pH regulation when using both BC and PY together. This also makes the BS more favorable for other beneficial microorganisms and promotes mechanisms which require a pH near 7 (Guardado 2019).

Other microorganisms such as *Arthrobacter* sp. ZXY-2, and various strains of the *Enterobacter*, *Pseudomonas*, *Bacillus* and *Providencia* genera have shown significant potential for atrazine removal (El-Bestawy *et al.* 2014; Zhao *et al.* 2018). A study conducted by El-Bestawy *et al.* (2014) demonstrated that seven bacterial species from the *Enterobacter*, *Pseudomonas*, *Bacillus* and *Providencia* genera demonstrated a high resistance to atrazine with high growth stimulation (70.7–88.7%) in atrazine-contaminated soil in addition to efficiently removing atrazine and its chlorinated derivatives (El-Bestawy *et al.* 2014). A beneficial synergistic effect was also observed from using a consortium of atrazine-resistant strains from the *Enterobacter*, *Pseudomonas*, *Bacillus* and *Providencia* genera, where greater atrazine removal was achieved compared to the atrazine removal achieved by the strains individually (El-Bestawy *et al.* 2014).

Various studies have demonstrated that the combination of a bioaugmentation and biostimulation approach yielded better results for atrazine removal as well as for other contaminants (El-Bestawy *et al.* 2014; Aliyu *et al.* 2023). Therefore,

bioaugmentation with a consortium of selected bacteria coupled with biostimulation may be the best bioremediation technique since it is an effective, low-cost and environmentally friendly approach for the decontamination of atrazine-contaminated matrices and may also be used to remove many other TrOCs (El-Bestawy *et al.* 2014; Aliyu *et al.* 2023).

Atrazine removal may be achieved via a hydrolytic pathway and in many cases, the process of mineralization forms traces of cyanuric acid (among other degradation products) (Monard *et al.* 2008; El-Bestawy *et al.* 2014; Aliyu *et al.* 2023). Also, Monard and coworkers have shown that *Pseudomonas* sp. ADP had a higher survival rate, when exposed to atrazine for 10 days, than *Chelatobacter heintzii* (Monard *et al.* 2008).

As for PCA production, findings from a study have shown that the presence of PY leads to the production of PCA, an antibiotic with a biopesticide effect, which could provide added value to treated sludge when used in agriculture (Arseneault *et al.* 2013). A study demonstrated over a 4-day period that a significant growth inhibition of *Botrytis cinerea* was only attained with a PCA concentration of at least 1.56 mg/L (Simionato *et al.* 2017). Since, in another study, the use of this PY strain alone was shown to have a significant effect on potato scabs and blight, it is reasonable to assume that using the same strain of PY or a similar one, can achieve higher concentrations of PCA than what was obtained after 90 days in this experiment (Arseneault *et al.* 2013; Morrison *et al.* 2017). Other strains of *Pseudomonas* spp. such as *P. aeruginosa* and *P. chlororaphis* have also been shown to have good potential for the production of PCA and have demonstrated an effectiveness in reducing and eliminating plant pathogens and promoting plant growth when used in soil (Jain & Pandey 2016; Simionato *et al.* 2017). It was confirmed that PY produces PCA (about 0.3 mg/L) as demonstrated in Figure 4. This is far less than the values presented in other studies for which a significant effect could be observed but PCA production could be increased if the culture parameters were optimized. It is also possible that a higher concentration was achieved during the 90 days and then gradually decreased to the measured concentration (as observed with the fluctuating enzymatic activity). A higher production of PCA could possibly be achieved with a higher initial concentration of PY or through a proper optimization process. However, the presence of BC reduced the production of PCA by a factor of five. This could indicate that adding PY to BC may not yield a high enough concentration of PCA to provide a significant biopesticide effect when applying the BS treated with PY and BC on agricultural fields. Further testing with optimized conditions would have to be done with pests and agricultural plants to determine the biopesticide efficiency of PY and BC combined vs. PY and BC on their own.

CONCLUSION

The PY strain studied produced significant concentrations of laccase (about 2,200 U/L), a ligninolytic enzyme useful for the removal of various TrOCs found in BS. PY also produced some quantities of lipase, amylase and phosphatase, which are also key enzymes for the treatment of BS. PY helps the existing bacterial flora in BS promote greater treatment efficiency by helping to remove the contaminants in BS. When PY is used in combination with BC, a synergistic effect can be observed where there is increased atrazine removal efficiency and seven times higher laccase production (about 15,000 U/L). Other studies have shown that laccase is a key enzyme involved in the degradation of many TrOCs such as atrazine and that bioaugmentation involving a consortium of TrOC-degrading microorganisms (including strains of the *Pseudomonas* and *Bacillus* genera) combined with biostimulation may be one of the most cost-effective ways to remove TrOCs such as atrazine from municipal BS, soil and water.

It was observed in this study that PY produces PCA (*circa* 300 μg/L in aqueous phase), although when combined with BC the yield was five times lower. Further tests involving agricultural plants and plant pathogens will be required to determine the biopesticide efficiency of PY and BC combined *versus* PY and BC on their own as well as the effect on plant growth.

It can be concluded from this study that PY has the potential to increase the efficiency of BC for the removal of atrazine. PY also produces PCA, which could help give a biopesticide effect to the sludge used on agricultural fields.

ACKNOWLEDGEMENTS

This project was supported by a grant from the National Science and Engineering Research Council of Canada (NSERC; grant number 2019-06178). The authors are thankful to Olivier Savary and the members of the Environmental Engineering Laboratory of the Université de Sherbrooke for their assistance and NUVAC Eco-Sciences Inc. for supplying *P. yamanorum* LBUM636 and BactoCharge.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

Ahmid, D. H. & Ismail, S. M. 2020 Effectiveness evaluation of Trichozone for *Trichoderma harzianum* and Fulzyme for *Bacillus subtillus* and *Pseudomonas fluorescens* in curbing causes of charcoal rot disease on the watermelon plant. *IOP Conf. Ser.: Earth Environ. Sci.* **553**, 012017. https://doi.org/10.1088/1755-1315/553/1/012017.

Aliyu, G. O., Anyanwu, C. U., Nnamchi, C. I. & Onwosi, C. O. 2023 Evaluation of the effectiveness of bioaugmentation and biostimulation in atrazine removal in a polluted matrix using degradation kinetics. *Soil Sediment Contam. Int. J.* **32**, 105–124. https://doi.org/10.1080/15320383.2022.2059444.

American Public Health Association, American Water Works Association, Water Environment Federation 2023 Lipps, W. C., Braun-Howland, E. B. & Baxter, T. E., eds. *Standard Methods for the Examination of Water and Wastewater*, 24th edn. APHA Press, Washington, DC.

Arseneault, T., Goyer, C. & Filion, M. 2013 Phenazine production by *Pseudomonas* sp. LBUM223 contributes to the biological control of potato common scab. *Phytopathology* **103**, 995–1000. https://doi.org/10.1094/PHYTO-01-13-0022-R.

Asif, M. B., Hai, F. I., Kang, J., Van De Merwe, J. P., Leusch, F. D. L., Price, W. E. & Nghiem, L. D. 2018 Biocatalytic degradation of pharmaceuticals, personal care products, industrial chemicals, steroid hormones and pesticides in a membrane distillation-enzymatic bioreactor. *Bioresour. Technol.* **247**, 528–536. https://doi.org/10.1016/j.biortech.2017.09.129.

Belal, E., Eissa, F., Zidan, N. & Nasr, I. 2013 Bioremediation of atrazine-contaminated water and soils by *Pseudomonas fluorescens*. *Int. J. Microbiol. Res.* **4**, 241–252. https://doi.org/10.5829/idosi.ijmr.2013.4.3.7687.

Daughton, C. G. 2010 Pharmaceutical ingredients in drinking water: overview of occurrence and significance of human exposure. In: *Pharmaceutical Ingredients in Drinking Water: Overview of Occurrence and Significance of Human Exposure*. American Chemical Society. Washington, DC, pp. 9–68. https://doi.org/10.1021/bk-2010-1048.ch002

Drouin, M., Lai, C. K., Tyagi, R. D. & Surampalli, R. Y. 2008 *Bacillus licheniformis* proteases as high value added products from fermentation of wastewater sludge: pre-treatment of sludge to increase the performance of the process. *Water Sci. Technol. J. Int. Assoc. Water Pollut. Res.* **57**, 423–429. https://doi.org/10.2166/wst.2008.007.

El-Bestawy, E., Sabir, J., Mansy, A. H. & Zabermawi, N. 2014 Comparison among the efficiency of different bioremediation technologies of atrazine-contaminated soils. *J. Biorem. Biodegrad.* **05**. https://doi.org/10.4172/2155-6199.1000237

El Fantroussi, S. & Agathos, S. N. 2005 Is bioaugmentation a feasible strategy for pollutant removal and site remediation? *Curr. Opin. Microbiol.* **8**, 268–275. Ecology and Industrial Microbiology/Edited by Sergio Sánchez and Betty Olson. Techniques/Edited by Peter J Peters and Joel Swanson. https://doi.org/10.1016/j.mib.2005.04.011.

Folin, O. 1929 Enzymatic assay of protease using casein as a substrate [WWW Document]. Sigma-Aldrich. Available from: https://www.sigmaaldrich.com/technical-documents/protocols/biology/enzymatic-assay-of-protease-casein-as-a-substrate.html (accessed 27 October 2020).

Gamez, R., Cardinale, M., Montes, M., Ramirez, S., Schnell, S. & Rodriguez, F. 2019 Screening, plant growth promotion and root colonization pattern of two rhizobacteria (*Pseudomonas fluorescens* ps006 and *Bacillus amyloliquefaciens* bs006) on banana cv. Williams (*Musa acuminata* Colla). *Microbiol. Res.* **220**, 12–20. https://doi.org/10.1016/j.micres.2018.11.006.

Guardado, A. L. P. 2019 *Enzymatic Degradation of Recalcitrant Pharmaceutical Micropollutants*. *PhD Thesis*, University of Montpellier.

Haroune, L., Cassoulet, R., Lafontaine, M.-P., Bélisle, M., Garant, D., Pelletier, F., Cabana, H. & Bellenger, J.-P. 2015 Liquid chromatography-tandem mass spectrometry determination for multiclass pesticides from insect samples by microwave-assisted solvent extraction followed by a salt-out effect and micro-dispersion purification. *Anal. Chim. Acta* **891**, 160–170. https://doi.org/10.1016/j.aca.2015.07.031.

Haroune, L., Saibi, S., Cabana, H. & Bellenger, J.-P. 2017 Intracellular enzymes contribution to the biocatalytic removal of pharmaceuticals by *Trametes hirsuta*. *Environ. Sci. Technol.* **51**, 897–904. https://doi.org/10.1021/acs.est.6b04409.

Jain, R. & Pandey, A. 2016 A phenazine-1-carboxylic acid producing polyextremophilic *Pseudomonas chlororaphis* (MCC2693) strain, isolated from mountain ecosystem, possesses biocontrol and plant growth promotion abilities. *Microbiol. Res.* **190**. https://doi.org/10.1016/j.micres.2016.04.017

Janusz, G., Pawlik, A., Sulej, J., Świderska-Burek, U., Jarosz-Wilkołazka, A. & Paszczyński, A. 2017 Lignin degradation: microorganisms, enzymes involved, genomes analysis and evolution. *FEMS Microbiol. Rev.* **41**, 941–962. https://doi.org/10.1093/femsre/fux049.

Johansen, A. & Olsson, S. 2005 Using phospholipid fatty acid technique to study short-term effects of the biological control agent *Pseudomonas fluorescens* DR54 on the microbial microbiota in barley rhizosphere. *Microb. Ecol.* **49**, 272–281. https://doi.org/10.1007/s00248-004-0135-2.

Kolekar, P. D., Phugare, S. S. & Jadhav, J. P. 2014 Biodegradation of atrazine by *Rhodococcus* sp. BCH_2 to *N*-isopropylammelide with subsequent assessment of toxicity of biodegraded metabolites. *Environ. Sci. Pollut. Res.* **21**, 2334–2345. https://doi.org/10.1007/s11356-013-2151-6.

Kross, B. C., Vergara, A. & Raeu, L. E. 1992 Toxicity assessment of atrazine, alachlor, and carbofuran and their respective environmental metabolites using microtox. *J. Toxicol. Environ. Health.* **37**, 149–159. https://doi.org/10.1080/15226514.2012.759528.

Kumar, V. V. & Rapheal, V. S. 2011 Induction and purification by three-phase partitioning of aryl alcohol oxidase (AAO) from *Pleurotus ostreatus*. *Appl. Biochem. Biotechnol.* **163**, 423–432. https://doi.org/10.1007/s12010-010-9050-9.

Monard, C., Martin-Laurent, F., Vecchiato, C., Francez, A. J., Vandenkoornhuyse, P. & Binet, F. 2008 Combined effect of bioaugmentation and bioturbation on atrazine degradation in soil. *Soil Biol. Biochem., Special Section: Enzymes in the Environment* **40**, 2253–2259. https://doi.org/10.1016/j.soilbio.2008.04.022.

Morrison, C. K., Arseneault, T., Novinscak, A. & Filion, M. 2017 Phenazine-1-carboxylic acid production by *Pseudomonas fluorescens* LBUM636 alters *Phytophthora infestans* growth and late blight development. *Phytopathology®* **107**, 273–279. https://doi.org/10.1094/PHYTO-06-16-0247-R.

Oberhofer, T. R. 1979 Growth of nonfermentative bacteria at 42°C. *J. Clin. Microbiol.* **10**, 800–804. https://doi.org/10.1128/jcm.10.6.800-804.1979.

Olicón-Hernández, D. R., González-López, J. & Aranda, E. 2017 Overview on the biochemical potential of filamentous fungi to degrade pharmaceutical compounds. *Front. Microbiol.* **8**. https://doi.org/10.3389/fmicb.2017.01792.

Rathankumar, A. K., SaiLavanyaa, S., Saikia, K., Gururajan, A., Sivanesan, S., Gosselin, M., Vaidyanathan, V. K. & Cabana, H. 2019 Systemic concocting of cross-linked enzyme aggregates of *Candida antarctica* Lipase B (Novozyme 435) for the biomanufacturing of rhamnolipids. *J. Surfactants Deterg.* **22**, 477–490. https://doi.org/10.1002/jsde.12266.

Rathankumar, A. K., Saikia, K., Nagarajan, K. T., Vaithyanathan, V. K., Vaidyanathan, V. K. & Cabana, H. 2020 Development of efficient and sustainable added-value products from municipal biosolids through an industrially feasible process. *J. Cleaner Prod.* **266**, 121749. https://doi.org/10.1016/j.jclepro.2020.121749.

Sheikhi, F., Roayaei Ardakani, M., Enayatizamir, N. & Rodriguez-Couto, S. 2012 The determination of assay for laccase of *Bacillus subtilis* WPI with two classes of chemical compounds as substrates. *Indian J. Microbiol.* **52**, 701–707. https://doi.org/10.1007/s12088-012-0298-3.

Simionato, A., Pérez Navarro, M., Abreu de Jesus, M., Barazetti, A., Silva, C., Simões, G., Balbi-Peña, M., Mello, J., Panagio, L., Almeida, R., Andrade, G. & De Oliveira, A. 2017 The effect of phenazine-1-carboxylic acid on mycelial growth of *Botrytis cinerea* produced by *Pseudomonas aeruginosa* LV Strain. *Front. Microbiol.* **8**, 1102. https://doi.org/10.3389/fmicb.2017.01102.

Singh, S., Kumar, V., Chauhan, A., Datta, S., Basit Wani, A., Singh, N. & Singh, J. 2018 Toxicity, degradation and analysis of the herbicide atrazine. *Environ. Chem. Lett.* **16**, 211–237. https://doi.org/10.1007/s10311-017-0665-8.

Singh, S., Kumar, V., Upadhyay, N. & Singh, J. 2020 The effects of Fe(II), Cu(II) and humic acid on biodegradation of atrazine. *J. Environ. Chem. Eng.* **8**, 103539. https://doi.org/10.1016/j.jece.2019.103539.

Spahr, S., Teixidó, M., Sedlak, L., G, D. & Luthy, R. 2020 Hydrophilic trace organic contaminants in urban stormwater: occurrence, toxicological relevance, and the need to enhance green stormwater infrastructure. *Environ. Sci. Water Res. Technol.* **6**, 15–44. https://doi.org/10.1039/C9EW00674E.

Tabatabai, M. A. & Bremner, J. M. 1969 Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* **1**, 301–307. https://doi.org/10.1016/0038-0717(69)90012-1.

Tonelli Fernandes, A. F., Braz, V. S., Bauermeister, A., Rizzato Paschoal, J. A., Lopes, N. P. & Stehling, E. G. 2018 Degradation of atrazine by *Pseudomonas* sp. and *Achromobacter* sp. isolated from Brazilian agricultural soil. *Int. Biodeterior. Biodegrad.* **130**, 17–22. https://doi.org/10.1016/j.ibiod.2018.03.011.

Touahar, I. E., Haroune, L., Ba, S., Bellenger, J.-P. & Cabana, H. 2014 Characterization of combined cross-linked enzyme aggregates from laccase, versatile peroxidase and glucose oxidase, and their utilization for the elimination of pharmaceuticals. *Sci. Total Environ.* **481**, 90–99. https://doi.org/10.1016/j.scitotenv.2014.01.132.

Vaithyanathan, V. K., Ravi, S., Leduc, R., Vaidyanathan, V. K. & Cabana, H. 2020 Utilization of biosolids for glucose oxidase production: a potential bio-Fenton reagent for advanced oxidation process for removal of pharmaceutically active compounds. *J. Environ. Manage.* **271**, 110995. https://doi.org/10.1016/j.jenvman.2020.110995.

Vaithyanathan, V. K., Cabana, H. & Vaidyanathan, V. K. 2021 Remediation of trace organic contaminants from biosolids: influence of various pre-treatment strategies prior to *Bacillus subtilis* aerobic digestion. *Chem. Eng. J.* **419**, 129966. https://doi.org/10.1016/j.cej.2021.129966.

Vaithyanathan, V. K., Vaidyanathan, V. K. & Cabana, H. 2022 Laccase-driven transformation of high priority pesticides without redox mediators: towards bioremediation of contaminated wastewaters. *Front. Bioeng. Biotechnol.* **9**, 770435. https://doi.org/10.3389/fbioe.2021.770435.

Vélez, J. M. B., Martínez, J. G., Ospina, J. T. & Agudelo, S. O. 2021 Bioremediation potential of Pseudomonas genus isolates from residual water, capable of tolerating lead through mechanisms of exopolysaccharide production and biosorption. *Biotechnol. Rep.* **32**, e00685. https://doi.org/10.1016/j.btre.2021.e00685.

Vieno, N., Tuhkanen, T. & Kronberg, L. 2007 Elimination of pharmaceuticals in sewage treatment plants in Finland. *Water Res.* **41**, 1001–1012. https://doi.org/10.1016/j.watres.2006.12.017.

Wang, J., Zhu, L., Wang, Q., Wang, J. & Xie, H. 2014 Isolation and characterization of atrazine mineralizing *Bacillus subtilis* strain HB-6. *PLoS ONE* **9**. https://doi.org/10.1371/journal.pone.0107270

Wattiau, P., Renard, M. E., Ledent, P., Debois, V., Blackman, G. & Agathos, S. N. 2001 A PCR test to identify *Bacillus subtilis* and closely related species and its application to the monitoring of wastewater biotreatment. *Appl. Microbiol. Biotechnol.* **56**, 816–819. https://doi.org/10.1007/s002530100691.

World Health Organization 2012 *Pharmaceuticals in Drinking-Water*. World Health Organization, Geneva.

Yang, S., Hai, F. I., Price, W. E., McDonald, J., Khan, S. J. & Nghiem, L. D. 2016 Occurrence of trace organic contaminants in wastewater sludge and their removals by anaerobic digestion. *Bioresour. Technol., Special Issue on Challenges in Environmental Science and Engineering (CESE-2015)* **210**, 153–159. https://doi.org/10.1016/j.biortech.2015.12.080.

Yang, J., Li, W., Ng, T. B., Deng, X., Lin, J. & Ye, X. 2017 Laccases: production, expression regulation, and applications in pharmaceutical biodegradation. *Front. Microbiol.* **8**, 832–856. https://doi.org/10.3389/fmicb.2017.00832.

Yu, S., Zhang, G., Li, J., Zhao, Z. & Kang, X. 2013 Effect of endogenous hydrolytic enzymes pretreatment on the anaerobic digestion of sludge. *Bioresour. Technol.* **146**, 758–761. https://doi.org/10.1016/j.biortech.2013.07.087.

Zhang, T., Qu, Z., Li, B. & Yang, Z. 2019 Simultaneous determination of atrazine, pendimethalin, and trifluralin in fish samples by QuEChERS extraction coupled with gas chromatography-electron capture detection. *Food Anal. Methods* **12**, 1179–1186. https://doi.org/10.1007/s12161-019-01449-z.

Zhao, X., Wang, L., Ma, F. & Yang, J. 2018 Characterisation of an efficient atrazine-degrading bacterium, *Arthrobacter* sp. ZXY-2: an attempt to lay the foundation for potential bioaugmentation applications. *Biotechnol. Biofuels* **11**, 113. https://doi.org/10.1186/s13068-018-1113-0.

Zhu, J., Fu, L., Jin, C., Meng, Z. & Yang, N. 2019 Study on the isolation of two atrazine-degrading bacteria and the development of a microbial agent. *Microorganisms* **7**. https://doi.org/10.3390/microorganisms7030080

First received 24 January 2022; accepted in revised form 29 March 2023. Available online 12 May 2023