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Comparison between two reactors using *Trametes versicolor* for agricultural wastewater treatment under non-sterile condition in sequencing batch mode

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ABSTRACT

Agricultural wastewater is a major source of herbicides, which pose environmental and health concerns owing to their substantial use and poor elimination rate in conventional wastewater treatment plants. White-rot fungi are versatile in degrading xenobiotics; however, the key problem encountered with their application in actual scenarios is competition with indigenous microorganisms, mainly bacteria. To address this barrier, two different strategies were implemented in the present study. One strategy was to set up a trickle bed with *Trametes versicolor* immobilized on pine wood, and another strategy was to employ a *T. versicolor*-pelleted, fluidized-bed reactor to remove diuron and bentazon from actual wastewater under non-sterile conditions. The residence time in the trickle bed was estimated using three methodologies. With 10 batches of a 3-day cycle operation, although the trickle-bed reactor possessed a shorter contact time (8.5 h per cycle) and lower laccase activity compared with those of the fluidized-bed reactor, it demonstrated a higher removal yield and lower bacterial counts. In addition, the utilization of pine wood as a carrier obviously reduced the cost since no additional nutrients were required. Hence, after evaluating all advantages and limitations of both bioreactors, for the purpose of treating over the long term and scaling up, a trickle-bed reactor is the preferred choice.

1. Introduction

In recent decades, diverse micropollutants that demonstrate persistence, partly because of their high volumes of production and consumption resulting from anthropogenic activities and partly because of their poor elimination rates in conventional wastewater treatment plants (WWTPs), have represented a severe global concern, and these micropollutants include pesticides, pharmaceuticals, personal care products, steroid hormones, industrial chemicals and many other emerging compounds (Luo et al., 2014). Among them, pesticides are considered the main trigger of environmental deterioration, although their use addresses the increasing food demand and population explosion (Meftaul et al., 2020; Rani et al., 2020).

As a key component of modern global agricultural systems, herbicide sales account for the largest proportion of agrochemicals and currently for more than 40% of their consumption (Carvalho, 2017). Diuron [3-(3, 4-dichlorophenyl)-1,1-dimethylurea], one type of phenylurea herbicide, is used extensively to control pre- and postemergence weeds for many agricultural crops, such as fruit, cotton, and sugar cane (Liu, 2014), while bentazon [3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2, 2-dioxide] is a thiadiazine herbicide and used largely postemergence in rice cultivation areas for the control of broadleaf weeds as well as sedges (Gillespie et al., 2011). Due to either recalcitrance (Giacomazzi and Cochet, 2004; Liu, 2014) or extended use (López-Piñeiro et al., 2017), both pollutants are ubiquitous in different water bodies (Barbieri et al., 2020a; Barbieri et al., 2021; Vieira et al., 2016; Wan et al., 2020) through leaching or runoff (Giacomazzi and Cochet, 2004; Hua et al., 2009; López-Piñeiro et al., 2017), and accumulating evidence shows that they pose multifaceted threats towards non-target organisms and ecosystems (Giacomazzi and Cochet, 2004; Liu, 2014; Macedo et al., 2008; Turcant et al., 2003). Hence, the development of techniques to address this concern is strongly encouraged and imperative.

In comparison with physical and chemical approaches, bioremediation is regarded as a low-cost, eco-friendly, and efficient alternative technique for the abatement of a broad range of pollutants (Azubuike et al., 2016). In particular, as a result of a battery of well-developed

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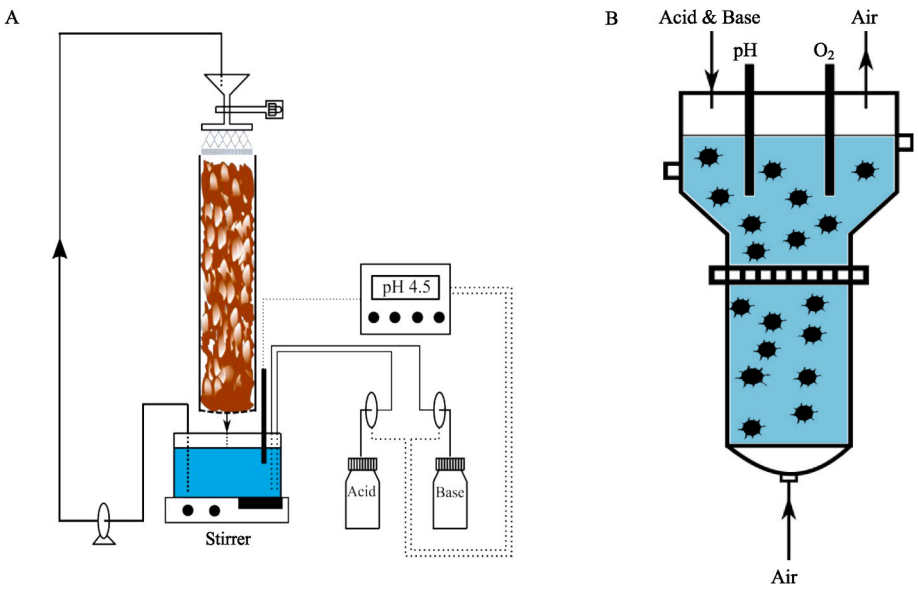

Fig. 1. Schematic representations of the trickle-bed reactor (A) and the fluidized-bed reactor (B) setup.

ligninolytic enzymes, white-rot fungi (WRF) are effective at degrading various recalcitrant contaminants (Mir-Tutusaus et al., 2018). The application of fungal reactors, especially fungal pelleted reactors, in wastewater treatment has been well documented (Espinosa-Ortiz et al., 2016). Our group has focused on air-pulsed fluidized-bed reactors (FBRs) using pellets of *Trametes versicolor* for years, and good results have been obtained for different xenobiotics under sterile conditions (Blánquez et al., 2007; Jelic et al., 2012). However, some issues appeared under non-sterile conditions, including bacterial contamination and biomass ageing (Mir-Tutusaus et al., 2018). Although several solutions have been proposed for long-term application, such as partial biomass renovation, pH adjustment or coagulation-flocculation pretreatment (Mir-Tutusaus et al., 2017), economically, FBR is still an expensive option. Notably, a trickle-bed reactor (TBR) with *T. versicolor* immobilized on pine wood was set up by Hu et al. (2020), and it could successfully remove diuron from tap water. However, a comparison of the performances of these two reactors in the scenario of pesticide-contaminated wastewater treatment is still unknown.

To address this gap, the objective of the present work was to set up a trickle-bed reactor and fluidized-bed reactor using *T. versicolor* as inoculum for agricultural wastewater treatment under non-sterile conditions and then to compare their performances and practicality from different perspectives. It is hoped that this work can provide perspectives for scaling-up approaches and long-term processes.

2. Materials and methods

2.1. Microorganism and medium

T. versicolor ATCC 42530 was acquired from the American Type Culture Collection and maintained by subculturing every 30 days on 2% (w/v) malt extract Petri dishes at 25 °C. Blended mycelial suspensions and pellets were prepared using malt extract as described by Blánquez et al. (2004).

For the TBR experiment, the packing material was prepared by inoculating a blended mycelial suspension into autoclaved pine wood (*Pinus* sp.) chips as reported by Torán et al. (2017), followed by 4 weeks of incubation at 25 °C.

2.2. Chemicals and regents

Diuron (purity, ≥ 98%) and 2,6-dymetoxyphenol (DMP, 99%) were purchased from Sigma-Aldrich (Barcelona, Spain). The commercial herbicide KAOS-B (bentazon, 48%) was obtained from SAPEC AGRO (Barcelona, Spain). D (+)-Glucose was purchased from Acros Organics (New Jersey, USA). Ammonium chloride was obtained from Scharlau (Barcelona, Spain). Chromatographic grade acetonitrile was purchased from Carlo Erba Reagents S.A. S (Barcelona, Spain). Formic acid (purity, ≥ 98%) was used as a mobile phase modifier, and ferric chloride was obtained from Merck (Darmstadt, Germany). PROSEDIM CS 209 was acquired from Degrémont Iberia (Bilbao, Spain). Stock solutions (10 mg mL^{-1}) of diuron and bentazon to be used for wastewater fortification were prepared by appropriate dilution of the substances in ethanol and stored in the dark at 4 °C until use.

2.3. Agricultural wastewater

Agricultural wastewater (AW) was collected from irrigation channels in Gavà, within the Llobregat River Basin (Catalonia, Spain), but during two different periods. The wastewater used for the TBR (AW I) was collected in February 2019, whereas that for the FBR (AW II) was obtained in August 2020. They were stored at 4 °C until use. In the case of AW II, a coagulation-flocculation pretreatment was applied to reduce algal biomass. Coagulant ferric chloride and flocculant PROSEDIM CS 209 were employed in this process, during which 2 min of coagulation at 200 rpm, 15 min of flocculation at 20 rpm and 30 min of settling were performed. Specifically, AW II was pretreated with 40 mg L^{-1} coagulant and 2 mg L^{-1} flocculant. The characteristics of the pretreated AW II and AW I are given in Table S1 of the supplementary material (SM).

2.4. Bioreactor setup

2.4.1. Trickle-bed reactor

As shown in Fig. 1A, a cylindrical methacrylate TBR (Ø 8.3 cm, H 59 cm) with an approximate working volume of 2.5 L and a porosity of 60% was set up. Essentially, 1 L of AW I fortified with diuron and bentazon to a final concentration of 10 mg L^{-1} for both pollutants was loaded into the packing bed from the top of the reactor through a rotary distributor and then collected by the reservoir tank placed at the bottom. The collected water was mixed by a magnetic stirrer, and its pH was maintained at 4.5 by adding either 1 M HCl or 1 M NaOH. An external bottom-to-top recirculation loop (flow rate 70 mL min^{-1}) was provided. Simultaneously, an identical reactor filled with non-colonized chips was operated in parallel to assess the removal contribution from

lignocellulosic supporting material. Multiple runs were implemented in sequencing batch mode at room temperature, and a 3-day cycle was adopted. Samples were taken from the tank after each batch to measure laccase, pesticide concentration, heterotrophic plate counts (HPCs) and chemical oxygen demand (COD), and then, the wastewater was totally replenished.

2.4.2. Fluidized-bed reactor

Pellets were transferred into a glass air-pulsed fluidized reactor, achieving the same biomass in terms of dry weight (DW) as that in the TBR. This reactor was designed with a cylindrical vertical centre body connected with a diametrically wider head (Fig. 1B). The useful volume of this FBR was 1.5 L, and it carried 1 L of AW II spiked with diuron and bentazon. The concentration was set to 10 mg L^{-1} for each herbicide. The reactor was operated in sequencing batch mode with a 3-day cycle at 25 °C. The pH was controlled at a constant value of 4.5 by the addition of 1 M HCl or 1 M NaOH through several ports at the top. Air was introduced from the bottom using an electrovalve through which the fluidized condition (1 s air pulse every 4 s) in the centre body was sustained. The aeration rate was approximately 0.8 L min^{-1}. Nutrients including glucose and NH_4Cl were fed with a molar C/N ratio of 7.5 based on the glucose consumption rate of *T. versicolor* (Mir-Tutusaus et al., 2017). At the end of each batch, samples were withdrawn for laccase, pesticide concentration, HPCs, COD and glucose analyses, and then, pellets were harvested and reinoculated to replenished wastewater.

2.5. Residence time of the TBR

Taking into account that the contact time between the immobilized fungi and the water has been suggested to play a vital role in the regime of a TBR (Hu et al., 2020), residence time determination is important for the employed reactor. In this work, the residence time was determined through three different techniques: (1) Volumetric quantification. After the wood was soaked with water, the pump was switched on for a certain time, and all the introduced distilled water was collected at the outlet. The time corresponding to collecting a 10 mL aliquot was recorded. (2) Pulse tracer injection. After injecting 3 mL of 3 M KCl at the top of the reactor, the effluent was collected every 15 s, and the conductivity was measured. (3) Step tracer input. The influent was changed to a KCl solution (46 mM) at a certain point, and then, the samples were collected and analysed analogously to the pulse input strategy. A flow rate of 70 mL min^{-1} was employed in all experiments, and each kind of test was conducted in duplicate.

2.6. Analytical procedures

2.6.1. Laccase

After filtration through a 0.22 μm hydrophilic polypropylene syringe filter (Scharlau, Barcelona, Spain), laccase activity was measured through the oxidation of DMP by the enzyme as described elsewhere (Wariishi et al., 1992). Activity units per L (AU L^{-1}) were defined as the amount of DMP in μM that was oxidized in 1 min. The molar extinction coefficient of DMP was 24.8 mM^{-1} cm^{-1}.

2.6.2. Pesticide residues

Samples were filtered using a Millipore Millex-GV unit equipped with a polyvinylidene difluoride (PVDF) membrane (0.22 μm) prior to analysis. The residual diuron and bentazon concentrations were determined through an HPLC (Ultimate 3000, Dionex, USA) equipped with a UV detector. Chromatographic analysis was achieved with a mobile phase consisting of 0.01% (v/v) formic acid in water (A) and acetonitrile (B) at a flow rate of 0.9 mL min^{-1} and a C18 reversed-phase column (Phenomenex®, Kinetex® EVO C18 100 Å, 4.6 mm × 150 mm, 5 μm) set at 30 °C. A gradient elution programme was performed as follows: the percentage of B was 35% from 0 min to 0.5 min, then linearly increased

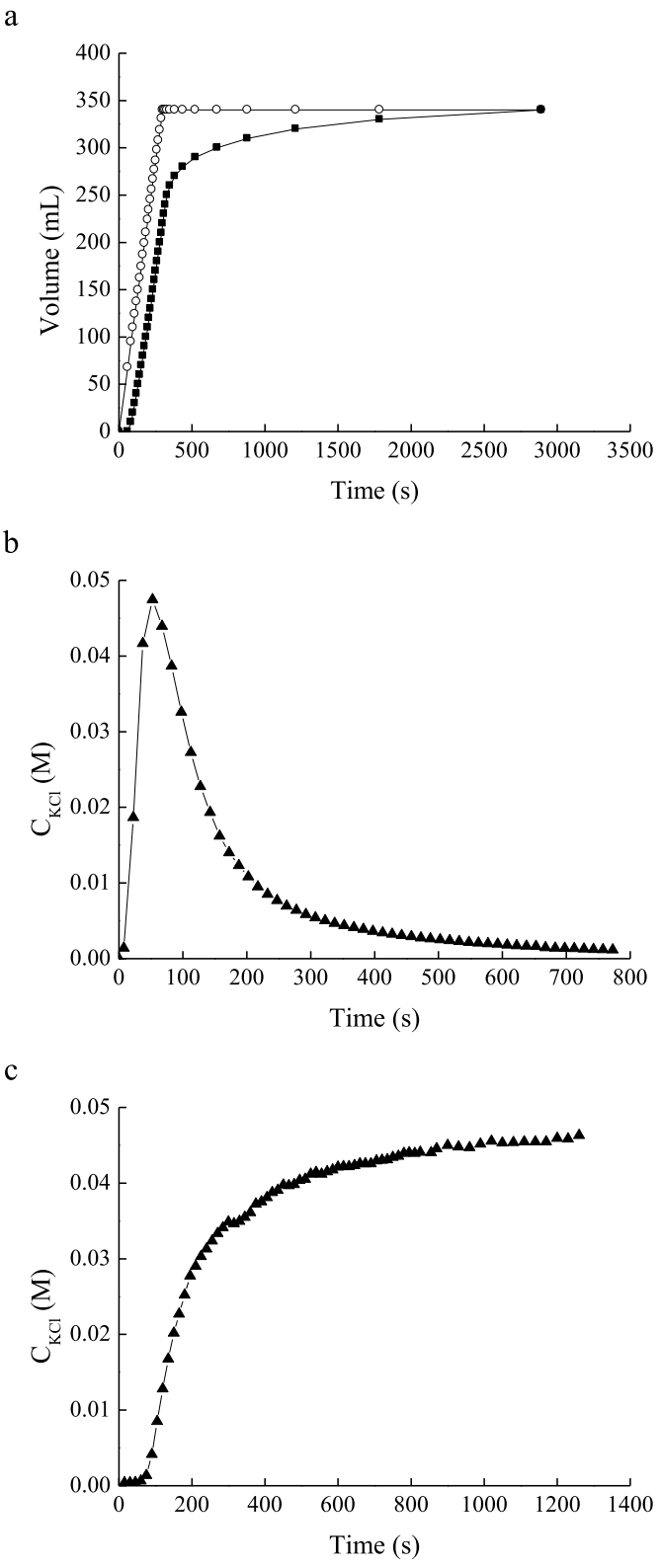

Fig. 2. Responses to TBR residence time determination using different techniques. a. volumetric quantification. b. pulse tracer injection. c. step tracer input. *Empty circles*, input volume; *filled squares*, output volume; *filled triangles*, tracer concentration at outlet.

Table 1
Average residence time of the TBR determined through different techniques.

Technique	Residence time (s)
Volumetric quantification	102 ± 2
Pulse tracer injection	173 ± 1
Step tracer input	259 ± 6

Note: Means and standard deviation of duplicates are shown.

to 45% from 0.51 min to 12 min, followed by decreasing to 35% in 1 min and maintaining 35% for 2 min. The injection volume was 40 μL. The detection wavelength was set at 254 nm, and a limit of detection of 0.5 mg L^{-1} was accomplished by using this analysis condition.

2.6.3. Biomass

Biomass in terms of pellets was determined by the dry weight, obtained after filtrating the culture and drying the residue at 105 °C to a constant weight.

With regard to the TBR, biomass was quantified by measuring ergosterol as previously described by Rodríguez-Rodríguez et al. (2010). However, the HPLC analysis method was slightly modified since a C18 reversed-phase column (Phenomenex®, Kinetex® EVO C18 100 Å, 4.6 mm × 150 mm, 5 μm) was adopted in the present study. The isocratic eluent was switched to acetonitrile (100%), and the retention time was 7.593 min.

2.6.4. Other analyses

The glucose concentration was measured using a biochemistry analyser (2700 select, Yellow Springs Instrument, USA) after filtrating the sample with a Millipore Millex-GV PVDF syringe filter (0.22 μm).

The absorbance at 650 nm was determined by a UNICAM 8625 UV/VIS spectrometer, and the conductivity was monitored by a CRISON MicroCM 2100 conductometer. The total suspended solids (TSSs) and volatile suspended solids (VSSs) were measured according to the standard methods 2540 D and 2540 E (Baird et al., 2017), respectively. The total organic carbon (TOC) was determined using an Analytik Jena multi N/C 2100 S/1 analyser. The HPC results were reported as the logarithm of colony-forming units (CFU) per mL [lg (CFU mL^{-1})] using the spread-plate method with a plate count agar (PCA) following the standard method 9215 (Baird et al., 2017). The N–NH_4^+ concentration and the COD were analysed by using the commercial kits LCK 303 and LCK 314 or LCK 114 or LCK 014 (Hach Lange, Germany). Chloride, sulfate, nitrite and nitrate anions were quantified by a Dionex ICS-2000 inorganic chromatograph equipped with a Dionex IonPac AS18-HC column (250 mm × 4 mm), which was eluted at 1 mL min^{-1} with a 13 mM KOH aqueous solution.

2.6.5. Data analysis

The degradation percentage was calculated using the following equation:

$$Removal\ percentage(\%) = \frac{M_0 - M}{M_0} \times 100\%$$

where M_0 corresponds to the initial amount of contaminant (mg), and M represents the residual amount of contaminant (mg).

3. Results and discussion

3.1. TBR residence time

To determine the residence time of the TBR, three different strategies were adopted in this study, and the responses and calculated results are presented in Fig. 2 and Table 1. For the volumetric quantification technique shown in Fig. 2a, the flow rate of the outlet became constant and equalled that of the inlet after 102 s, which can be assumed to be the

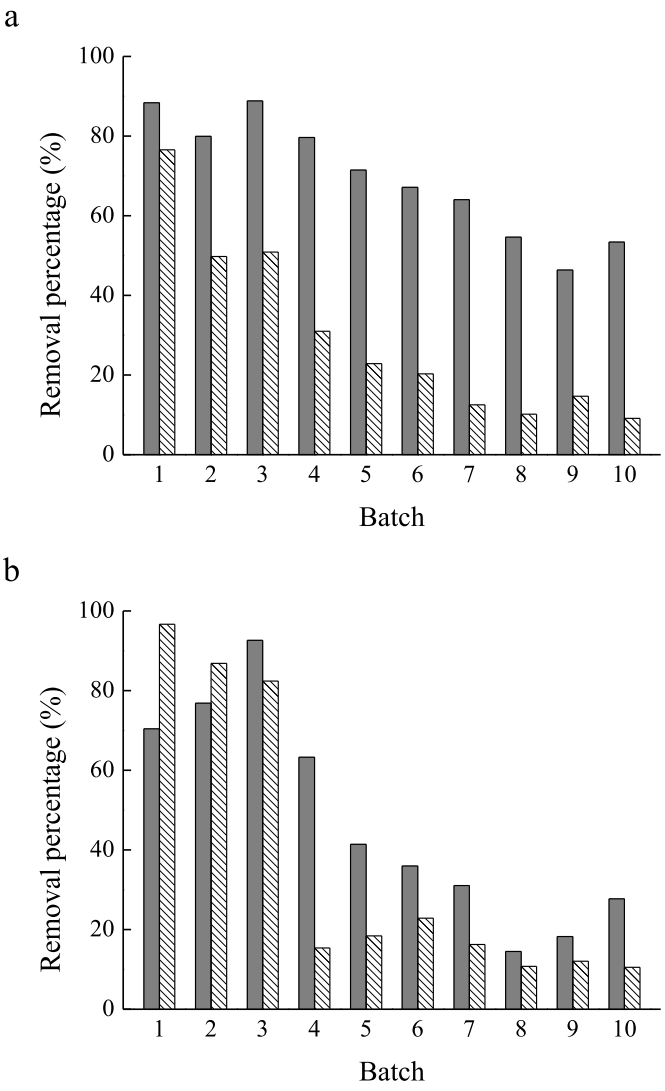

Fig. 3. Profiles of pesticides removal by the two different fungal reactors in sequencing batch mode under non-sterile conditions. a. diuron; b. bentazon. *Gray columns*, trickle-bed reactor; *medium columns*, fluidized-bed reactor.

average time required for the liquid to pass through the reactor, namely, the residence time. However, this characteristic changed to significantly higher values when pulse input and step input were used (Table 1) since the conductivity proportional responses were not symmetric and the curves contained long tails (Fig. 2b and c). Similar profiles were obtained by Sá and Boaventura (2001) using siliceous granular material to support the biomass in the TBR. A reasonable explanation could be that part of the packing bed was relatively stagnant due to the irregular shape of the wood chips, for which even a rotary distributor had been provided at the top of the reactor. The main water flow went downwards through preferred routes, while a small amount of tracer was retained in the reactor and left very slowly. Therefore, it can be concluded that the TBR employed in this work did not perform as an ideal plug-flow reactor. However, if the relatively stagnant volume (tails) was ignored, as mentioned by Levenspiel (2002), 102 s corresponds to the time at which half of the mass of 80% of the tracer injection (active volume) was collected in the scenario of pulse input and to the moment when half of the volume of flow in the rector was substituted by tracer solution in the scenario a step input. Hence, we consider that among the residence times, 102 s was the most representative residence time. Given that an operation recirculation rate of 300 (recirculation flow rate at 70 mL min^{-1}) was applied in the present study, the total contact time was

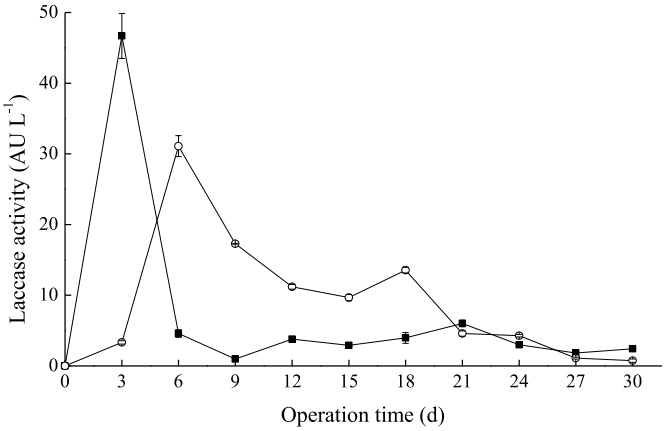

Fig. 4. Profile of laccase activity during operation. *Filled squares*, trickle-bed reactor; *empty circles*, fluidized-bed reactor.

actually 8.5 h for each batch.

3.2. Pesticide removal performance in the two different reactors

Several pieces of colonized wood chips were used before operation to measure the initial biomass in the TBR, and the ergosterol concentration was approximately 0.044 ± 0.002 mg per g of dry wood. Considering that 367 g of dry chips was introduced and the ergosterol content of the selected strain corresponded to 6.61 mg gDW^{-1} of fungal biomass (Rodríguez-Rodríguez et al., 2010), we determined that the total biomass of the TBR was approximately 2.45 g DW. Hence, an equivalent pelleted inoculum was transferred into the FBR for operation.

As shown in Fig. 3, considerable removals of diuron and bentazon occurred in the TBR, yielding 69.38% and 47.21% removal on average, respectively; in contrast, the FBR was less effective, at 29.79% and 37.22% removal, respectively. However, the elimination performance was similar between the two reactors at the beginning of operation, and this performance was even better for bentazon in the FBR than in the TBR. This result could be explained by the fact that although both sets employed a 3-day cycle, the contact time between the fungi and pollutant was quite low in the case of the TBR based on the above empirical calculations (8.5 h per cycle). Therefore, the contribution of the cytochrome P450 system of *T. versicolor* to the selected pesticide degradation was restricted due to the configuration of the TBR, and this particular intracellular enzyme is largely involved in diuron and bentazon degradation (García-Vara et al., 2020; Hu et al., 2020). On the other hand, bearing in mind that the lignocellulosic-supporting material in the TBR, namely, pine wood chips, played an indispensable role in the treatment owing to its adsorption since the results from the control reactor showed that 83.22% of diuron was removed and 28.02% of bentazon was removed; these values were 25.75% and 8.47%, respectively, in regard to pellets in an Erlenmeyer test (García-Vara et al., 2020; Hu et al., 2020). Such apparent differences between the two pesticides could be ascribed to their different hydrophobicities according to the Pesticide Properties Database (https://sitem.herts.ac.uk/aeru/ppdb/en/index.htm). However, regardless, the presence of lignocellulosic carriers enhances and maintains the capability of reactors to eliminate target pesticides from water bodies, which is consistent with the results of a more recently published study where a great extent of pharmaceuticals (73.3%) were adsorbed by the bed that was mainly composed of rice husks (Tormo-Budowski et al., 2021). This evidence further indicates that these organic packing materials play a vital role in wastewater treatment using a TBR. Additionally, it is worth noting that a decreasing trend in the removal yield was observed in both reactors, probably ascribed to the fungal ageing behaviour and biomass washing out.

Table 2
HPC and COD of the agricultural wastewater throughout the sequencing batch treatment by the two different fungal reactors.

Batch	Microbial counts [Log (CFU) mL^{-1}]		COD (mg L^{-1})	
	TBR	FBR	TBR	FBR
1	4.44	8.23	671	1172
2	2.90	8.90	590	1188
3	0	8.76	521	1706
4	3.90	8.52	438	2107
5	6.00	8.24	382	1695
6	6.10	7.85	318	1815
7	6.13	7.20	280	1696
8	5.99	7.29	288	2097
9	5.92	7.79	208	2565
10	5.87	7.95	248	2611

Note: TBR, trickle-bed reactor; FBR, fluidized-bed reactor.

3.3. Comparison between the trickle-bed reactor and air-pulsed fluidized-bed reactor

Clearly, the TBR demonstrated better results than the FBR based on the contaminant removal yield, as mentioned above, but this evidence is not strong enough to determine which fungal reactor is an ideal option. Therefore, attention was also paid to bioactivity persistence in the two systems. Laccase activity was monitored during operation because it partly reflects fungal activity (Mir-Tutusaus et al., 2017), and the results are shown in Fig. 4. An enzymatic activity peak of 47 AU L^{-1} was achieved in the TBR at the end of first batch, probably resulting from accumulation within the static incubation, whereas this peak occurred in second running period of the FBR, at 31 AU L^{-1}. Then, although laccase activity displayed a decreasing pattern in both setups and eventually remained at a low constant level, its variation was obviously slower in the FBR than in the TBR. The primary reasons behind this observation could be the different nutrient conditions in the reactors and the possibility that extracellular laccase was adsorbed onto the wood (Arora and Gill, 2000; Li et al., 2018). Furthermore, adequate oxygen transfer can be guaranteed by employing air-pulse conditions, while air supply should be included in future work using TBRs.

In addition, the HPC and COD of the wastewater after treatment by the trickle-bed reactor and fluidized-bed reactor are presented in Table 2. A dramatic increase in the bacterial amount was observed in the FBR compared that in the raw water. This result could be explained by the continuous feeding of glucose [1200 mg glucose (gDW d)$^{-1}$] for maintenance (Mir-Tutusaus et al., 2017), and the supplemented glucose was almost fully consumed in each batch (final concentration < 0.1 g L^{-1}). With respect to the TBR, although the microbial growth also accelerated after 4 batches, it was largely hindered initially, especially during the 3rd batch, appearing as having no microbial count. In addition, bacterial contamination remained at a lower level in the TBR than in the FBR, indicating that utilization of pine wood as a supporting material is a successful strategy to limit bacterial growth, and this represents a bottleneck in fungal reactor application regimes (Mir-Tutusaus et al., 2018). At the same time, the total COD increased in the two treatments compared with that in the initial wastewater. A common reason may be that the spiked pesticides were not completely addressed. This result could also be explained by the elution of wood particles from the packed bed and/or wood rotting by *T. versicolor* in the TBR, whereas the contribution was from the addition of antifoam (Tween 80) in the FBR. Fortunately, foam formation was not a concern for the trickle-bed reactor treatment since it is somewhat an open-scale system.

Regardless, future efforts need to focus economic costs while moving to a scaling-up process. Apart from preparing mycelial suspensions, there is an extra process before starting fluidized-bed reactor treatments compared to starting trickle-bed reactors, that is, pellet formation. Although a low-cost procedure using a defined medium instead of a malt extract to culture fungi was developed to obtain pellets and can satisfy a

Table 3
Advantages and limitations of the two reactor configurations for pesticides contaminated wastewater treatment in sequencing batch mode under non-sterile conditions.

Reactor type	Advantages	Limitations
Trickle-bed	● Simple construction and great operational flexibility ● Retain biomass in the reactor ● Hinder bacterial growth ● Low-cost and high sustainability ● No nutrients required ● Nature conditions emulated	● Short contact time ● Low mass transfer ● Poor oxygen supply ● Risk of clogging in long term operation ● Lack of homogeneity ● High pumping energy
Fluidized-bed	● High homogeneity ● Adequate cellular retention time ● Efficient mass transfer	● Required pellet preparation process ● Continuous nutrient supply ● Higher bacterial competition ● Foam formation ● Pellet break-up or aggregation ● Complicated separation of biomass ● Biomass renovation for long term operation

large demand (Borràs et al., 2008), other raw materials still bring cost concerns, such as the addition of maintenance nutrients and antifoam, which subsequently escalate bacterial contamination and increase COD. Another point of concern is that a partial biomass renovation strategy should be implemented in the case of long-term treatment, and the cost of heating energy resulting from sterilization during biomass preparation is reasonably higher than the mechanical energy that was necessary for recirculation with a pump. In addition, other problems related to pellet break-up appeared during operation, resulting in biomass loss because of adherence to the bioreactor wall and difficulties in separating biomass from the liquid phase during emptying. In contrast, the TBR is a relatively more robust configuration. Pine wood performs two functions as a carrier and a source of nutrients, not only taking advantage this reusable waste but also building a natural aerial habitat in which *T. versicolor* demonstrates effectiveness. Moreover, the immobilized fungi demonstrated persistent activity, and the wood was not visibly degraded during the whole process, indicating that a reinoculation strategy can be taken into consideration to enhance and maintain removal effectiveness. Based on the above information, the advantages and limitations of these two reactors are summarized in Table 3.

4. Conclusions

Good diuron and bentazon removal yields were obtained with the trickle-bed reactor and fluidized-bed reactor operating in a 3-day cycle sequential batch mode inoculated with *T. versicolor* to treat agricultural wastewater for 30 days under non-sterile conditions. A simple methodology was proposed to establish a representative average residence time, which has been compared to classical methodologies. After assessing the advantages and limitations of both configurations, for the purpose of long-term treatment and scaling up, a trickle-bed reactor is the preferred choice based on the evaluation of different aspects, and increasing residence time and biomass is the main concern.

Credit author statement

Kaidi Hu: Investigation, Writing original draft, Editing. Montserrat Sarrà: Conceptualization, Supervision and Reviewing. Gloria Caminal: Conceptualization, Supervision and Reviewing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2021.112859.

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