

The operating performance of a biotrickling filter with *Lysinibacillus fusiformis* for the removal of high-loading gaseous chlorobenzene

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Abstract Removal of gaseous chlorobenzene (CB) by a biotrickling filter (BTF) filled with modified ceramics and multi-surface hollow balls during gas–liquid mass transfer at the steady state was by microbial degradation rather than dissolution in the spray liquid or emission into the atmosphere. The BTF was flexible and resistant to the acid environment of the spray liquid, with the caveat that the spray liquid should be replaced once every 6–7 days. The BTF, loaded with *Lysinibacillus fusiformis*, performed well for purification of high-loading CB gas. The maximum CB gas inlet loading rate, $103 \text{ g m}^{-3} \text{ h}^{-1}$, CB elimination capacity, $97 \text{ g m}^{-3} \text{ h}^{-1}$, and CB removal efficiency, 97.7 %, were reached at a spray liquid flow rate of 27.6 ml min^{-1} , an initial CB concentration of up to $1,300 \text{ mg m}^{-3}$, and an empty bed retention time of more than 45 s.

Keywords Biotrickling filter · Biofilms · Chlorobenzene · Gaseous chlorobenzene · Gas–liquid mass transfer · *Lysinibacillus fusiformis*

Abbreviations

BTF	Biotrickling filter
CB	Chlorobenzene
C_{in}	The CB gas inlet concentration
C_{out}	The CB gas outlet concentration
EBRT	The empty bed retention time
EC	The CB elimination capacity
ILR	The CB gas inlet loading rate
Q	The CB gas inlet flow rate
RE	The CB removal efficiency
VOCs	Volatile organic compounds
v	The spray liquid flow rate

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Introduction

Prolonged exposure to chlorobenzene (CB) contamination has mutagenic, teratogenic and carcinogenic effects on human health (Field and Sierra-Alvarez 2008). Therefore, the control and treatment of CB pollutants is important. The conventional treatment methods for CB pollutants include physical, chemical and biological processes. Physical and chemical processes, such as adsorption (Liu et al. 2011; Zhao et al. 2001), condensation (Huang et al. 2013), and photolytic degradation (Zhang and

Anderson 2013), etc., often require strict operating conditions, a certain dose of other chemicals, and complex response configurations. Therefore, biological methods have become the focus of research efforts due to their low cost, simple operation, and low secondary pollution. Compared with biofilters and bioscrubbers, biotrickling filters (BTFs) offer flexible control of the spray liquid, pH, and intermediate toxic products, and have obvious advantages in the degradation of inorganic waste gases, such as ammonia (Lopez et al. 2013; Xue et al. 2010) or a variety of volatile organic compounds (VOCs) (Nicoletta et al. 2009; Lebrero et al. 2012). However, the application of a BTF to CB containing waste gases has been rarely reported and basic data related to this process is lacking.

Microorganisms are the key factors that determine whether BTF systems are running well (Yang et al. 2010). The common microbial agents used in the BTF include: a single dominant species, mixed species, or decomposer communities of single- and mixed-species. Under the different process conditions, each type of agent displays different degradation efficiencies. At present, the research into the predominant strains that degrade VOCs gives preference to artificially domesticated strains and mostly focuses on bacteria.

The choice of packing materials in the BTF is also crucial (Liu and Wang 2012). Packing materials with high robustness, high porosity, large specific surface area, good hydrophilicity, high surface roughness, and moderate grain size are the most suitable for microbial attachment and gas–liquid mass transfer, and have advantages in resisting any drop in pressure.

In this report, one dominant high concentration CB-degrading strain, *Lysinibacillus fusiformis* LW13 (Li et al. 2013), was activated and cultured for amplification. It was then used to form biofilms on the packing materials in the BTF. During the stable operation of the BTF, the accumulation of intermediates and pH changes in the spray fluid were monitored and analyzed. The CB gas outlet concentration (C_{out}), the CB gas inlet loading rate (ILR), the CB elimination capacity (EC), and the CB removal efficiency (RE) were evaluated while varying the spray liquid flow rate (v), the CB gas inlet concentration (C_{in}), the CB gas inlet flow rate (Q), and the empty bed retention time (EBRT).

These results will provide a starting point for future in-depth studies, and industrial applications, of CB waste gas removal by a single dominant species in the BTF.

Materials and methods

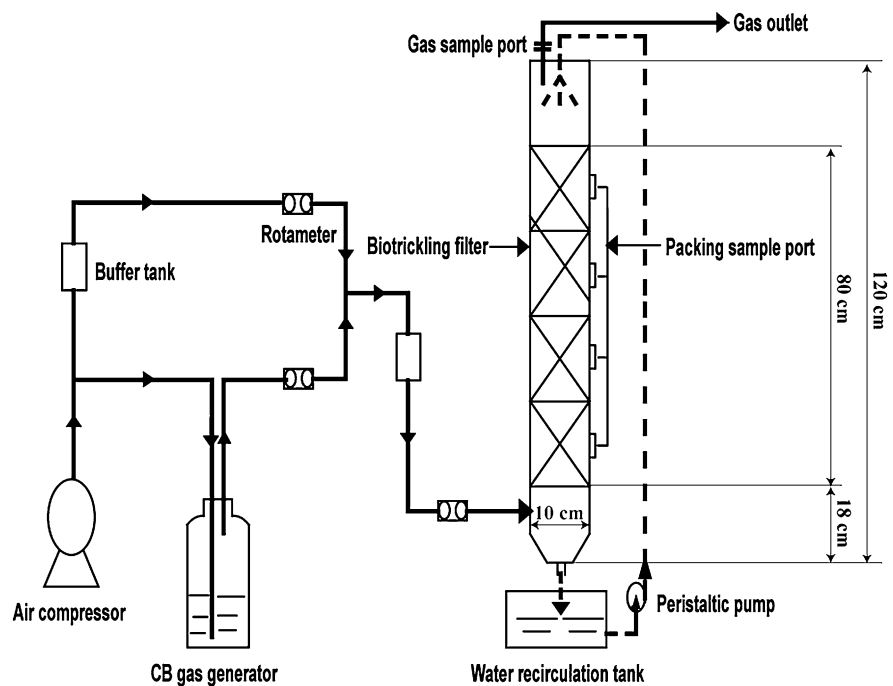
Materials

The packing material of the BTF was a mixture of modified ceramics, 1.2 cm × 1.5 cm, and multi-faceted hollow balls, diam. 1.5 cm. The two packing materials were inert to chlorobenzene (CB) absorption and were randomly mixed at a ratio of modified ceramics: multi-surface hollow balls = 1–1.5:1. The inoculated strain, *Lysinibacillus fusiformis* LW13 (GenBank accession number JN166076), was pre-screened with a high concentration of CB, and its ability to use CB as its sole carbon source was preserved in our laboratory. The sterilized mineral medium without the carbon source (Li et al. 2013) was used as the spray liquid and prepared before use.

Schematic diagram and operation of the BTF

The BTF was made of Plexiglass, diam. 10; 120 cm (Fig. 1). The packing layer (total ht 80 cm; total volume 6.28 l) was divided by five porous clapboards into four semi-continuous separate units. The diam. of each pore was 0.8 cm and the pores were uniformly aligned on each clapboard at intervals of 0.4 cm. A rotation axis was inserted through the clapboards at the center and rotated at 40 rpm to ensure good gas–liquid mass transfer. The spray liquid, sealed in a water recirculation tank, was pumped through a peristaltic pump to the top of the BTF in a countercurrent operation and was then evenly sprayed through a sprinkler on the surface of the packing materials. The liquid CB was sealed to prevent evaporative losses to the atmosphere and boiled in a water-bath along with driving of an air flow from the air compressor to form CB gas, which flowed through a rotometer and mixed with another air flow from the air compressor to obtain the simulated waste gas. The CB loading could be controlled over an appropriate range by varying the ratio of the gas flow rate of the two rotometers. The tests were carried out under atmospheric pressure at 25 ± 2 °C. Q was 0.25–0.6 m³ h⁻¹, C_{in} was

Fig. 1 Schematic diagram of the biotrickling filter (BTF)



277–1,670 mg m^{-3} , and v was 7.88–47.4 ml min^{-1} , the corresponding EBRT was 37–90 s, and ILR was 15.7–146.18 $\text{g m}^{-3} \text{h}^{-1}$. The spray liquid was refreshed once every cycle period during a 7 d cycle.

Determination of the CB concentration

The CB concentration was determined using head-space GC. Three parallel determinations were made for each sample and the average value was used. The peak area of the samples' standard curve was a linear function of the CB concentration. An Elite-5 capillary column (30 m \times 0.32 mm \times 0.5 μm) was used. The volume of the splitless injection was 500 μl , and it was injected into the vaporization chamber at 200 $^{\circ}\text{C}$. The column flow was 1.5 $\text{ml N}_2 \text{min}^{-1}$ and the column temperature program was: 70 $^{\circ}\text{C}$ for 1 min, an increase to 110 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C min}^{-1}$, and a hold at 110 $^{\circ}\text{C}$ for 1 min. The detector (FID) was at 250 $^{\circ}\text{C}$.

Determination of pH value and accumulation of metabolites

The pH of the spray liquid was measured with a pH meter. The accumulation of metabolites was evaluated at $A_{255\text{nm}}$ (Seigneur et al. 2002).

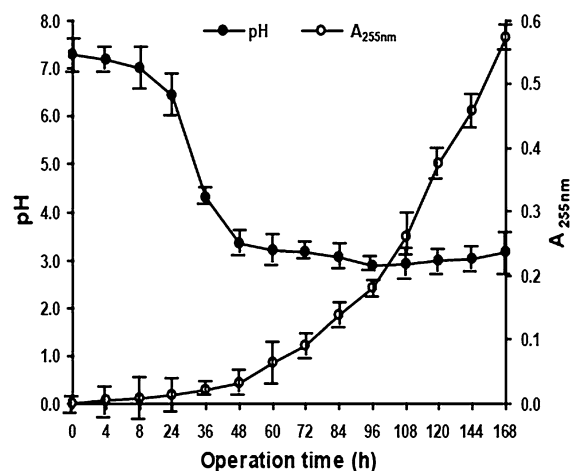


Fig. 2 pH and $A_{255\text{nm}}$ in the BTF within a cycle period

Results and discussion

pH and intermediate products in the BTF within a cycle period

In the BTF, the dominant degradation strain produces HCl during the CB biodegradation process causing the system to become acidic, which can, in turn, affect the CB levels. CB biodegradation also produces a variety

of metabolites, the accumulation of which can affect growth and even have a toxic effect on the dominant degradation strain. The BTF was run for one cycle under conditions of a v of 30 ml min^{-1} , C_{in} of $1,200 \text{ mg m}^{-3}$, and an EBRT of 75 s from a corresponding Q of $0.3 \text{ m}^3 \text{ h}^{-1}$. To examine the effect on the CB levels as a function of pH value and the accumulation of metabolites, the pH value of the spray liquid and its $A_{255\text{nm}}$ (Seigneur et al. 2002) were monitored over time (Fig. 2).

Within 60 h, the pH of the spray liquid decreased rapidly from an initial pH of 7.3 to pH 3.2. It then fluctuated around pH 3. This demonstrated that the spray liquid became acidic and that the microorganisms in the BTF could adapt quickly and resist the acidic environment of the spray liquid. The $A_{255\text{nm}}$ of the spray liquid could not be detected until 96 h, and then constantly increased to 0.574 at 168 h. This indicated that, as shown in Supplementary Fig. 1, the metabolites gradually accumulated as the BTF was in operation and were negatively related to the CB purifying effect. Therefore, in the CB purification process, the pH of the spray liquid should not be adjusted as an attempt to maintain neutral conditions. Rather, to maintain and maximize the utility of the nutrients in the spray liquid, it should be replaced once every 6–7 days.

Amount of CB in the spray liquid and BTF within a cycle period

It is possible that a small amount of CB could be removed from the BTF by the spray liquid when the liquid is replaced. To determine this, the CB concentration in the spray liquid was continuously monitored within a cycle period, at the same time points when the pH and intermediate products in the BTF were determined. The results showed that the amount of CB in the spray liquid averaged 78 mg with only minor fluctuations after being in operation for 2 days (Fig. 3). This was well below the average cumulative reduction of CB, which was 40.4 g in the BTF in a single cycle. This illustrates that there were small levels of soluble and accumulated CB in the spray liquid. C_{out} , shown in Supplementary Fig. 1a, indicated that the average CB loss from the gas outlet to the atmosphere was 3.2 g . Thus, the removal of CB was mostly due to the biological function of the dominant CB-

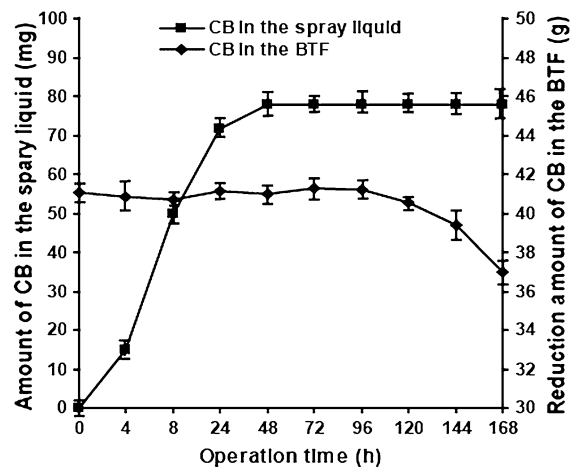


Fig. 3 Amount of chlorobenzene in the spray liquid and the BTF within a cycle period

degradation strain. Microstructures of the packing materials with biofilms formed by the dominant CB-degradation strain (Supplementary Fig. 2) and the CB levels (Supplementary Fig. 1) also demonstrated that the BTF system was at a steady operational state.

CB levels as affected by C_{in}

As CB was the sole carbon source in the BTF, C_{in} would play a key role in normal microbial growth and metabolism. When v was maintained at 30 ml min^{-1} , the removal of CB was investigated at different C_{in} of 0.25 , 0.4 , and $0.6 \text{ m}^3 \text{ h}^{-1}$ corresponding to EBRTs of 90 , 56 , and 37 s , respectively (Figs. 4, 5).

At a constant v and increasing C_{in} , at a number of different Q or EBRTs, there were consistent trends in C_{out} , ILR , EC and RE . When C_{in} was increased, C_{out} , ILR and EC gradually increased, but RE decreased significantly. This indicates that C_{in} had a significant impact on the CB levels. Because of the reduction in the biodegradation capacity, the biodegradability of CB in the BTF decreased and the increase in C_{out} was larger than the change in C_{in} . Therefore, EC was negatively correlated with RE . Thus, the CB levels could not be evaluated by simply using just EC or RE . Because of the assumption that C_{out} had to meet the integrated emission standard of air pollutants of China (CA-IES), C_{in} was kept under $1,300 \text{ mg m}^{-3}$ to improve EC and RE within this constraint.

Fig. 4 C_{out} and ILR at different C_{in} and EBRTs. C_{out} = chlorobenzene (CB) gas outlet concentration; ILR is the CB gas inlet loading rate; C_{in} is the CB gas inlet concentration; EBRT is the empty bed retention time

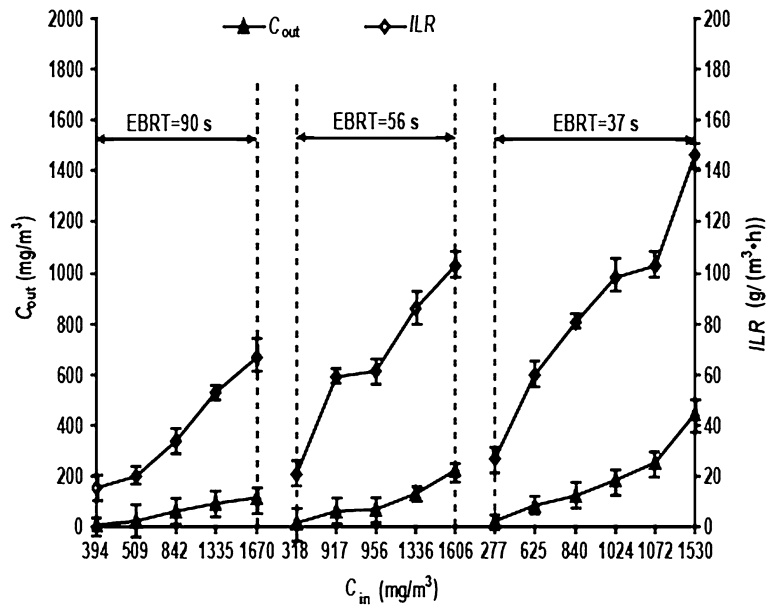
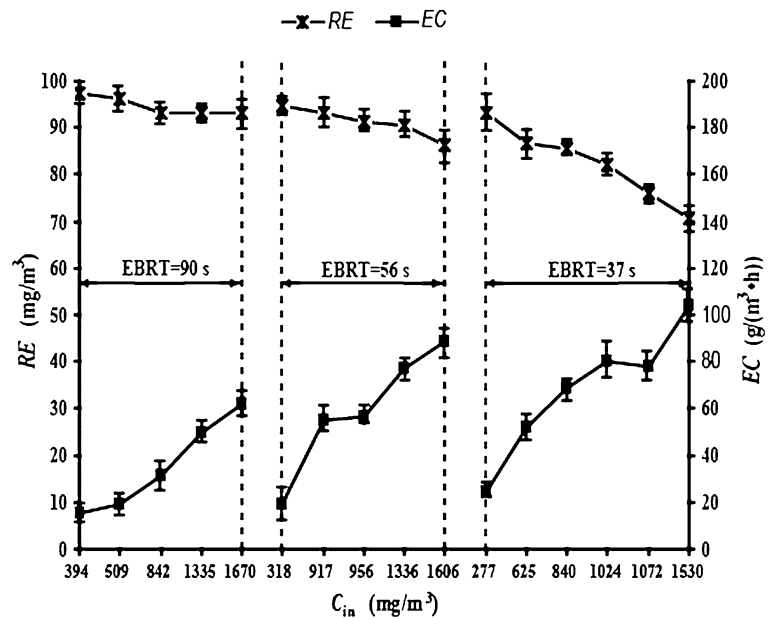


Fig. 5 RE and EC at different C_{in} and EBRTs. RE = chlorobenzene (CB) removal efficiency; EC = CB elimination capacity; C_{in} = CB gas inlet concentration; EBRT = empty bed retention time



CB levels as affected by the EBRT (empty bed retention time)

EBRT is also an important parameter to control during the operation of the BTF. Mass transfer between the microorganisms and the packing materials is low if the EBRT is too short, and the BTF will operate inefficiently if the EBRT is too long. Given a fixed BTF volume, there is a linear, positive correlation between

the EBRT and Q . Therefore, the CB levels as a function of EBRT were investigated at EBRTs of 90, 75, 56, 45, and 37 s (corresponding to Q of 0.25, 0.3, 0.4, 0.5, and 0.6 $m^3 h^{-1}$, respectively), at a constant v of 30 $ml min^{-1}$ and a C_{in} of 1,250 $mg m^{-3}$ (Figs. 6, 7).

At a constant v , there was a correlation between the EBRT or Q and C_{in} , C_{out} , ILR , EC and RE . As the EBRT was decreased by increasing Q , C_{in} , C_{out} , ILR

Fig. 6 C_{in} , C_{out} and ILR at different EBRTs.

C_{in} = chlorobenzene (CB) gas inlet concentration; C_{out} = CB gas outlet concentration; ILR = CB gas inlet loading rate; EBRT = empty bed retention time

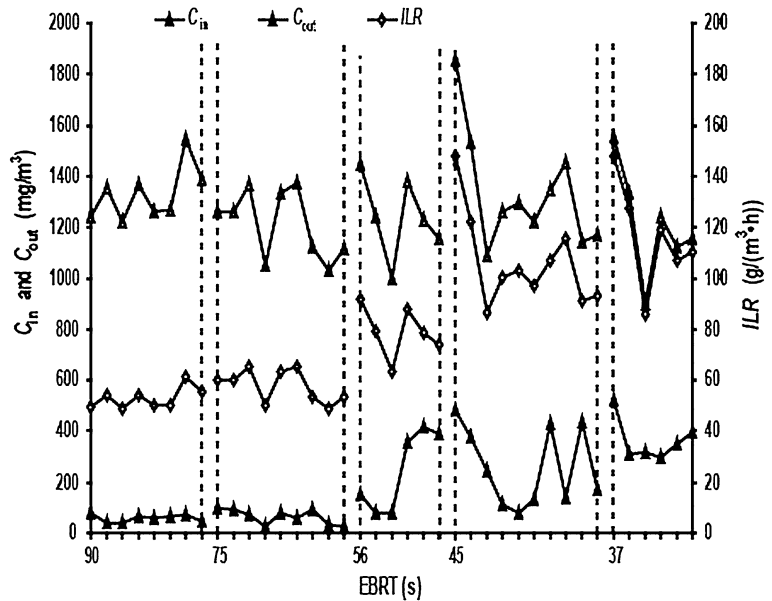
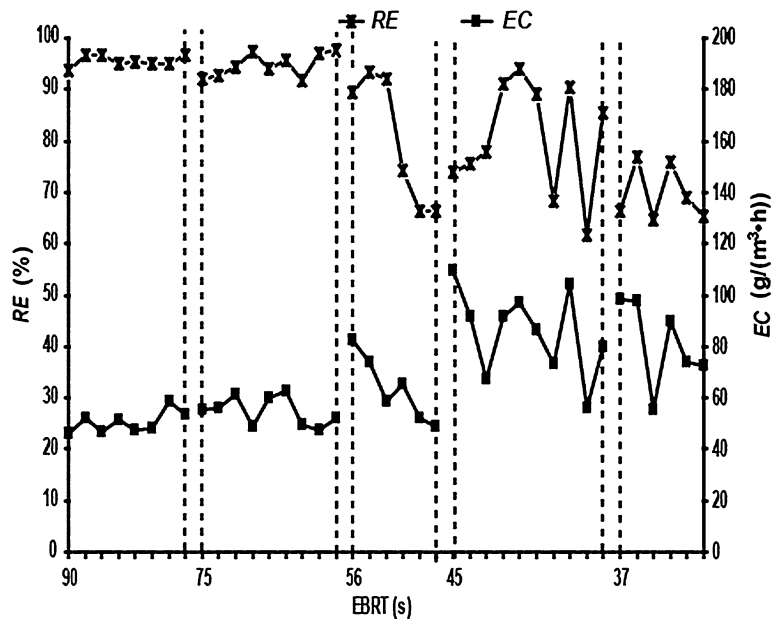


Fig. 7 RE and EC at different EBRTs.

(RE) = chlorobenzene (CB) removal efficiency; EC = CB elimination capacity; EBRT = empty bed retention time



and EC remained constant before increasing. These were all relatively large changes, although the RE was constant at first and then decreased. This shows that the EBRT or Q has a significant impact on the CB levels. At an EBRT of greater than 56 s (corresponding Q of $< 0.4 \text{ m}^3 \text{ h}^{-1}$), C_{in} , C_{out} , ILR , EC and RE all fluctuated over a small range. When the EBRT was $< 56 \text{ s}$ (or Q exceeded $0.4 \text{ m}^3 \text{ h}^{-1}$), C_{in} , C_{out} , ILR , EC and RE severely fluctuated over a large range,

particularly at the point where the EBRT or Q was initially changed. This could be due to limitations of the test device, where changes in the EBRT could change C_{in} . When the EBRT was further decreased, C_{in} was very large, so that the ILR of the BTF was affected by both the EBRT and C_{in} . Therefore, to ensure that C_{out} meets the CAIES and to improve the operational efficiency of the BTF, the EBRT should be no $< 45 \text{ s}$ from a corresponding Q of $< 0.5 \text{ m}^3 \text{ h}^{-1}$.

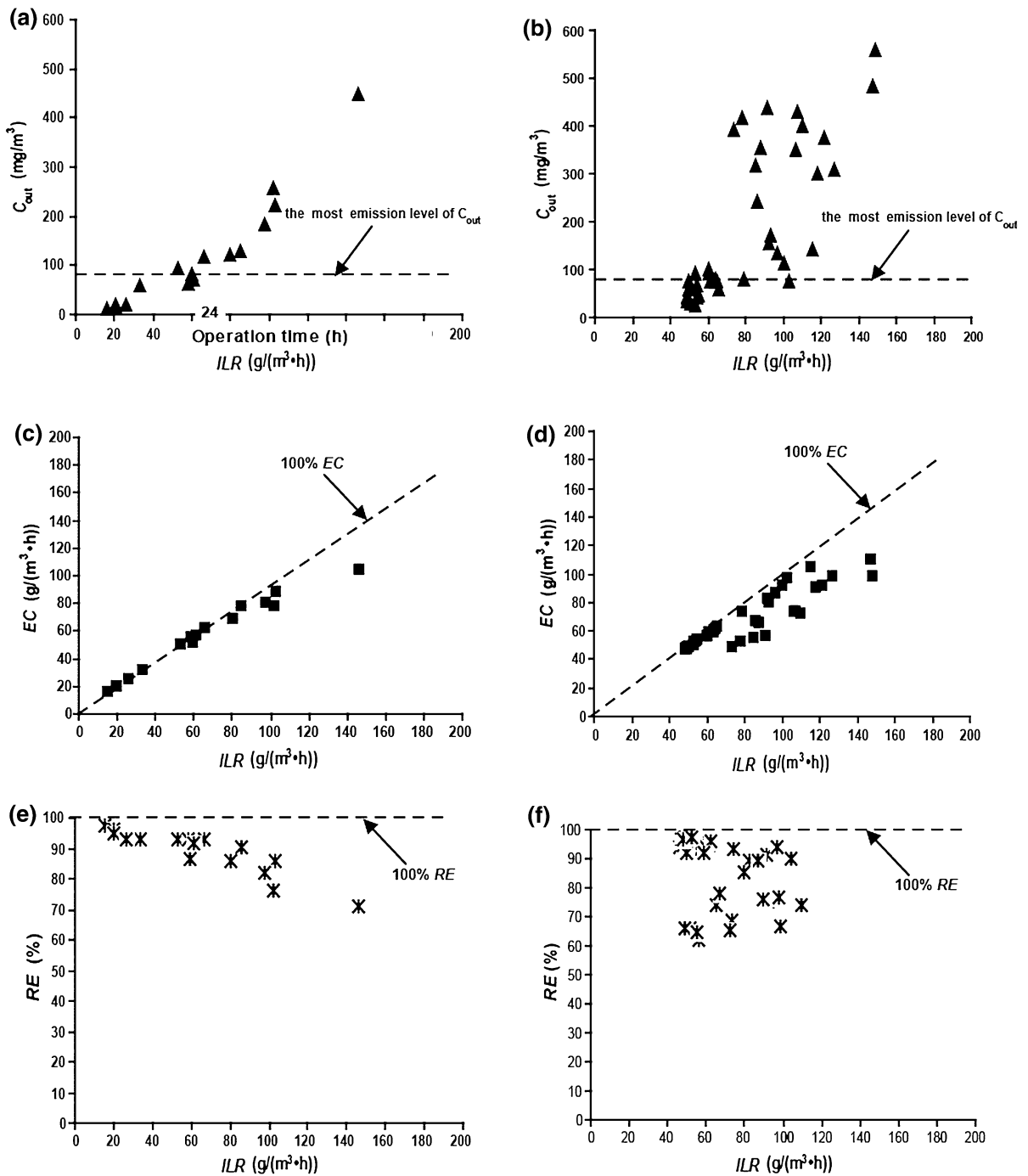


Fig. 8 C_{out} , EC and RE versus ILR **a** C_{out} versus ILR when C_{in} was varied. **b** C_{out} versus ILR when the EBRT was varied. **c** EC versus ILR when C_{in} was varied. **d** EC versus ILR when the EBRT was varied. **e** RE versus ILR when C_{in} was varied. **f** RE

versus ILR when the EBRT was varied C_{out} = chlorobenzene (CB) gas outlet concentration; EC = CB elimination capacity; RE = CB removal efficiency; ILR = CB gas inlet loading rate

CB levels as affected by ILR

Gas purification processes are controlled by the gas–liquid mass transfer rate and the biodegradation rate. At low pollutant loads, the biodegradation rate was larger than the mass transfer rate and therefore the process was controlled by the mass transfer rate. However, at high pollutant loads, the biodegradation rate was less than the mass transfer rate, so the process was controlled by the biodegradation rate. *ILR* depends on both Q and C_{in} . Therefore, a further investigation of the CB levels, as affected by *ILR*, was performed (Fig. 8).

When *ILR* was increased, C_{out} and *EC* increased significantly but *RE* still decreased. This means that *ILR* had a more significant impact on the CB levels in the BTF than did v . At low *ILR*, C_{out} could be maintained to meet the CAIES with a *RE* of more than 90 % and a linear, positive correlation between *EC* and *ILR*, which indicated that the mass transfer process and the biodegradation process were working well. When *ILR* was gradually increased, C_{out} exceeded this value, and *EC* and *RE* increasingly deviated from 100 % removal efficiency. This was probably because the mass transfer process became blocked and the biodegradation process began to play the predominant role. Thus, when the *ILR* was lower, the degradation of CB was closer to 100 %. When the *ILR* was higher, the total degradation of CB was limited by the mass transfer and biodegradation capacity of the BTF. Figure 8 also demonstrates that the distribution of C_{out} , *EC* and *RE* at different EBRTs was more discrete and more disordered than at different C_{in} . This indicated that the EBRT had a greater impact on *ILR* than C_{in} , which indirectly affected the CB levels of the BTF. To ensure that C_{out} meets CAIES, the maximum *ILR* of the BTF can be as high as $103 \text{ g m}^{-3} \text{ h}^{-1}$ with a maximum *EC* of $97 \text{ g m}^{-3} \text{ h}^{-1}$ and a maximum *RE* of 97.7 %, by adjusting v , the EBRT, and C_{in} .

In conclusion, a BTF with biofilms of the dominant degradation strain, *Lysinibacillus fusiformis* LW13, stably ran and eliminated significant amounts of CB in the BTF. This study demonstrated a particular advantage in treating high-loading gaseous CB with this setup as compared with using acclimated sludge (Zhou et al. 2011) or another single dominant species (Zhang et al. 2011). v (Supplementary Fig. 3), C_{in} , the EBRT and *ILR* affected the CB levels, with the three latter factors displaying the most significant effects. The

microorganisms in the BTF adapted to, and resisted, the acidic environment of the spray liquid, while the levels of metabolic intermediates could be monitored and used as a replacement signal for the spray liquid. Of the three indicators, C_{out} , *EC* and *RE*, that reflect the BTF's CB purification performance, C_{out} was the most direct and sensitive.

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Conflict of interest The authors declare that they have no conflict of interest.

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