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Energy, resources and environmental technology

Toluene degradation by a water/silicone oil mixture for the design of Two Phase Partitioning Bioreactors

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Highlights

- Toluene degradation was studied in a Two-Phase Partitioning Bioreactor (TPPB)
- The liquid phase consisted of 75 % water and 25 % silicone oil (PDMS 50)
- Biodegradation rates reached $104 \text{ g m}^{-3} \text{ h}^{-1}$ for 100 % toluene removal efficiency
- A TPPB for toluene removal can therefore be designed for large-scale applications

Abstract

Toluene degradation performances were studied in a 10 L Two-Phase Partitioning Bioreactor (TPPB). The liquid phase consisted of a mixture of water and PDMS 50 (PolyDiMethylSiloxane, i.e. silicone oil, viscosity of $46 \text{ mPa}\cdot\text{s}$) in the volume ratio of 75 %/25 %. Two series of experiments were carried out: in the first, the reactor was sequentially supplied with toluene whereas in the second, toluene was continuously supplied. Activated sludge from the wastewater treatment plant of Beaurade (Rennes, France) was used at an initial concentration of $0.5 \text{ dry mass g} \cdot (\text{mixture L})^{-1}$. The Elimination Capacity (EC) was investigated as well as the change in biomass concentration over time. Toluene biodegradation was very efficient (Removal Efficiency, $\text{RE} = 100 \%$) for toluene flows ranging from 0.2 to $1.2 \text{ ml} \cdot \text{h}^{-1}$, corresponding to elimination capacities of up to $104 \text{ g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$. For a toluene flow of $1.2 \text{ ml} \cdot \text{h}^{-1}$, the biomass concentration measured at the end of the experiment was $4.7 \text{ dry mass g} \cdot (\text{mixture L})^{-1}$. The oxygen concentration in the liquid phase was clearly not a limiting factor in these operating conditions. Based on these results, an extrapolation leading to the design of an large-scale pilot TPPB can now be considered to study toluene degradation performances in industrial conditions.

Keywords: Degradation; Toluene; Silicone oil; Multiphase reactor; Biomass.

1. Introduction

Atmospheric emissions of Volatile Organic Compounds (VOCs) represent an important environmental and human health issue. Biological treatment of VOC industrial emissions is particularly interesting due to its good performances obtained at low cost. However, traditional bioprocesses can be inadequate for removing hydrophobic VOCs as not only are such compounds usually poorly soluble in water, they can also be toxic for microorganisms. Toluene is a favorite hydrophobic model VOC among researchers because it is considered as an environmental priority pollutant and human carcinogen. Using a Two-Phase Partitioning Bioreactor (TPPB) could be an attractive alternative to remove such pollutants. A TPPB involves two immiscible liquid phases: an aqueous phase containing microorganisms and nutrients, and a Non-Aqueous Phase Liquid (NAPL) able to solubilize large amounts of hydrophobic VOCs. The targeted VOC is gradually transferred from the NAPL to the aqueous phase to be degraded by the microorganisms present in the TPPB, which thus enables the NAPL to be regenerated [1] while avoiding toxicity effects on the microorganisms. The VOCs can be removed from the air flux by absorption in a separate gas-liquid contactor before entering the TPPB (two-stage unit) or by direct blowing into the TPPB (one-stage unit). A hybrid system can also be considered [2]. Several review papers have highlighted the pros and cons of this technology [3–7]. Since the 2000s, some key scientific and technical limitations have gradually been solved. Thus, the selection of the most appropriate NAPL (i.e. immiscible with water, not biodegradable, not toxic for microorganisms and showing high affinity for hydrophobic VOCs) has been extensively studied [3,7,8]. To date, silicone oils (polydimethylsiloxane, PDMS) with a viscosity ranging from 20 to 200 mPa·s, appear to be the only NAPLs with the desired characteristics [7]. Once the best NAPL had been selected, efforts were focused on the determination of the gas/liquid partition coefficients between the target VOCs and silicone oils [1,9–15]. Moreover, optimization of the volume fraction of silicone oil needed for an efficient mass transfer in the gas-liquid contactor was considered [16,17] as well as the contactor hydrodynamics [2,18]. Simultaneously, much research was centered on mathematical modeling to determine the most important parameters governing VOC mass transfer and kinetic biodegradation [19–25]. Nevertheless, although significant data reported in the literature seem to demonstrate that TPPBs could be satisfactorily used at large-scale, no test *in situ* in real conditions has yet been carried out because some design issues remain to be solved. Indeed, the design of a large-scale TPPB is still not possible because there is no sufficient reliable data concerning the VOCs degradation performances in the presence of silicone oil. The results reported in the literature are extremely varied. Data can differ by one or two order of magnitude for the same VOC. For instance, studying the treatment of toluene as representative VOC in presence of silicone oil (PDMS 5 with a viscosity of 5 mPa·s), Darracq et al. [26] reported an elimination capacity (EC) of $0.95 \text{ g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ (25 % v/v of PDMS 5 in the mixture) whereas Littlejohns and Daugulis [27] reported an EC of $52 \text{ g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ (10 % v/v of PDMS 5 in the mixture). A toluene elimination capacity of $75 \text{ g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ (Removal Efficiency (RE) of 75 %) was obtained by Volckaert et al. [28] in the case of the treatment of a mixture of dimethylsulfide, hexane and toluene by a mixture of water/PDMS 20 (viscosity of 20 mPa·s) with a ratio of (75/25 v/v). The use of PDMS in stirred TPPBs has also been studied for the biodegradation of hexane, identified as a very hydrophobic pollutant. Elimination capacities of $21 \text{ g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ [29] and $60 \text{ g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ [30] were obtained using mixtures of water/PDMS 200 (viscosity of 200 mPa·s) with ratios of (90/10 v/v) and (80/20 v/v), respectively. Using a (75/25 v/v)

mixture of water/PDMS 20 for the treatment of hexane, Volckaert et al. [28] obtained elimination capacities up to $242 \text{ g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ (RE = 69 %). Such performances are lower than those reported by Montes et al. [31] for the biodegradation of α -pinene (a moderately hydrophobic VOC). Thus, using water/PDMS 200 mixtures with ratios of (98/2 v/v) and (95/5 v/v), these authors obtained RE = 100 % for loading rates up to around $100 \text{ g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ and reported a maximum elimination capacity of around $650 \text{ g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ (RE = 55 %). This short overview of data in the recent literature highlights that comparing results obtained for different VOCs is not really relevant. Even if the comparison is informative, the hydrophobicity of the VOC as well as its toxicity towards microorganisms and the mass transfer limitations have to be taken into account. Consequently, with the final objective to implement a full TPPB for the treatment of air polluted with toluene under industrial conditions, there is a need to determine the ability of microorganisms contained in a water/PDMS mixture to degrade this VOC. The purpose of this study was therefore to obtain useful data from the determination of toluene degradation performances by activated sludge in order to design an industrial TPPB. On the basis of “dimensional analysis”, the experiments carried out in a semi-continuous stirred tank reactor at laboratory-scale will be used to design and build a large-scale apparatus. In this study, toluene was selected as targeted VOC because it is largely used and emitted by many industries. Moreover, toluene is considered by the Total Company, partner of this project, as a compound of interest for the development of TPPBs.

2. Material and methods

2.1. Chemicals

Toluene (C_7H_8 ; CAS number: 108-88-3; purity ≥ 99.5 %; molecular weight: $92.14 \text{ kg} \cdot \text{mol}^{-1}$; density: $867 \text{ kg} \cdot \text{m}^{-3}$; Sigma Aldrich) was selected as the target VOC because it is widely used in various industries and is highly hydrophobic. The silicone oil Rhodorsil 47V50 (PDMS 50; dynamic viscosity $46 \text{ mPa} \cdot \text{s}$; density $959 \text{ kg} \cdot \text{m}^{-3}$), provided by the Bluestar Silicones Company, was selected based on characteristics such as its affinity for toluene (partition coefficient at 25°C : $2.9 \pm 0.3 \text{ Pa} \cdot \text{m}^3 \cdot \text{mol}^{-1}$; [14], non-biodegradability, biocompatibility and low aqueous solubility [1]. As the partition coefficient of toluene between water and air is $680 \text{ Pa} \cdot \text{m}^3 \cdot \text{mol}^{-1}$ at 25°C [32], it can be calculated that the affinity of toluene is 234 times higher for PDMS 50 than for water.

2.2. Experiments

Two sets of experiments were carried out in a semi-continuous stirred tank reactor (Fig. 1). The tank was aerated by a gas sparger and stirred by a Rushton turbine ($300 \text{ r} \cdot \text{min}^{-1}$). In the first set of experiments, the reactor was sequentially supplied with toluene (10 toluene injections) whereas in the second series, toluene was supplied continuously. Liquid toluene was injected into the mixture of water/PDMS 50 using a syringe driver. The operating conditions for all experiments are detailed in Table 1. Liquid temperature and pH were regulated at 25°C and 7, respectively. The liquid phase (10 L) consisted of 75 % water and 25 % silicone oil (PDMS 50) in volume corresponding to an optimum ratio for biodegradation performances [26]. The volume of the gas phase (head-space) was 2.3 L. Taking the stirring rate and the bubbling due to the aeration system into account, both the liquid and gas phases could be reasonably considered to be perfectly mixed. Activated sludge from the wastewater treatment plant of Beaurade (Rennes, France) was used at an initial concentration of $0.5 \text{ dry mass g} \cdot (\text{mixture L})^{-1}$ (i.e. $0.38 \text{ dry mass g} \cdot (\text{water L})^{-1}$). Nutrients were added to the reactor at the beginning of

experiments (all concentrations in $\text{g} \cdot (\text{water L})^{-1}$: KH_2PO_4 : 3.5; $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$: 8; NH_4Cl : 5.5; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.25; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$: 0.07; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$: 0.02; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$: 0.005; $(\text{NH}_4)_2\text{MoO}_7 \cdot 24\text{H}_2\text{O}$: 0.004; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$: 0.1). Since it was not regulated, biomass accumulated in the reactor during experiments. According to [33], half of the toluene degraded by the biomass is converted to cellular mass ($\text{C}_7\text{H}_8 + 4\text{O}_2 + \text{NH}_4^+ \rightarrow \text{C}_5\text{H}_7\text{O}_2\text{N} + 2\text{CO}_2 + 2\text{H}_2\text{O} + \text{H}^+$) and half is oxidized for energy ($\text{C}_7\text{H}_8 + 9\text{O}_2 \rightarrow 7\text{CO}_2$). Consequently, the amount of oxygen required to biodegrade 1 mole of toluene corresponds to 6.5 moles (i.e. 4.5 moles for energy production and 2 moles for biomass production). Moreover, it was recently evidenced that the presence of silicone oil has no significant influence on the microbial community in terms of richness and diversity [34].

Table 1 Operating conditions for the two sets of experiments

Toluene supply	Operating conditions	Measured parameters
Sequentially	Air flow rate: $Q_{\text{air}} = 60 \text{ air L} \cdot \text{h}^{-1}$ (residence time: 10 min) Toluene injection: $10 \text{ ml} \cdot \text{d}^{-1}$ at day 1, 3, 4, 5, 6, 7, 10, 11, 12, 13	Liquid phase: - O_2 dissolved - Biomass concentration Gas phase output: - Toluene concentration (C_{gas}) - O_2 concentration - CO_2 concentration
Continuous	Air flow rate: $Q_{\text{air}} = 45 \text{ air L} \cdot \text{h}^{-1}$ (residence time: 13 min) Toluene injection ($\text{ml} \cdot \text{h}^{-1}$): 0.2; 0.4; 0.6; 0.8; 1.0; 1.2	

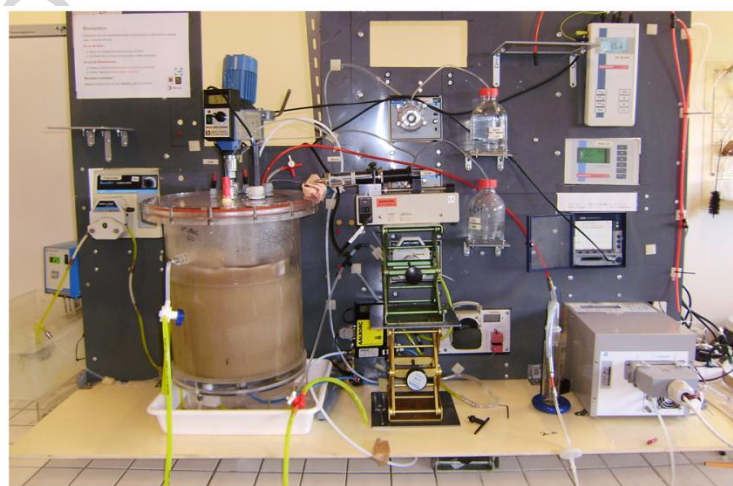
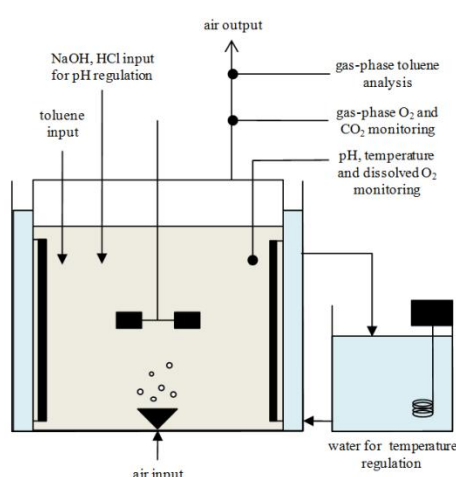


Figure 1 Semi-continuous 10 L stirred tank reactor for toluene biodegradation

2.3. Analytical methods

Biomass concentration was measured by extracting suspended solids from samples of the water/PDMS mixture by centrifugation (at 4000 rpm for 20 min) and weighing the dry matter (dried in an oven at 105°C for 16 hours). However, this measurement was difficult to make accurately because after centrifugation, on the one hand, a small part of the sludge was removed with the supernatant and, on the other hand, some traces of silicone oil remained “stuck” to the dry matter, distorting the weight values obtained (error of $\pm 20\%$). This issue is inherent to the use of water/NAPL mixtures. In fact, Ascon-Cabrera and Lebeault [35] observed that approximately half of the total biomass adhered to the water/NAPL interface. For the sequential experiments, biomass measurements were carried out daily, before toluene injection. For the continuous experiments, biomass

measurements were carried out at the end of each experiment.

The oxygen and carbon dioxide in the output gas were monitored simultaneously and continuously using an IPOS analyzer (Abiss, France). The dissolved oxygen in the aqueous phase was also monitored using a standard electrode SZ10T-PB (Consort, Belgium).

The toluene phase concentration in the gas phase was measured using a gas chromatograph (GC) coupled with a flame ionization detector (FID) from Thermo Scientific (USA) as described by Darracq et al. [36]. Assuming that gas-liquid equilibrium was reached in the reactor, the toluene concentration in the water/PDMS 50 mixture was then deduced from the partition coefficient value determined from the calculation procedure developed by Dumont et al. [12]. For a liquid phase consisting of 75 % water and 25 % PDMS 50 in volume, the partition coefficient value is $11.5 \text{ Pa} \cdot \text{m}^3 \cdot \text{mol}^{-1}$, corresponding to a dimensionless value of 0.0046 ($= C_{\text{gas}}/C_{\text{mixture}}$).

2.4. Biodegradation rate

For the sequential experiments, the toluene degradation rate (EC in $\text{g m}^{-3} \text{ h}^{-1}$) was calculated using Eq. (1):

$$\text{EC} = \frac{1}{V_{\text{mixture}}} \frac{\text{Mass of injected toluene} - \text{toluene stripping}}{\text{Degradation time}} \quad (1)$$

The volume of the mixture (V_{mixture}) was 10 L and the amount of injected toluene was 10 ml. The overall toluene stripping in the gas output during the whole experiment was deduced by monitoring the toluene concentration in the gas phase over time. Moreover, the degradation time was calculated from normalized curves ($n(t)/n(t=0)$) describing the decrease in the toluene content in the liquid phase over time, as well as from the oxygen concentration curves in the liquid and gas phases monitored during the course of experiments, respectively.

For the continuous experiments, the toluene biodegradation rate was calculated based on the mass balance between the toluene flow rate, toluene stripping, toluene degradation and toluene accumulation, as described in Eq. (2) and Fig. 2.

$$Q_{\text{toluene}} = Q_{\text{air}} C_{\text{gas}} + \text{degradation}_{(t)} V_{\text{mixture}} + V_{\text{mixture}} \frac{dC_{\text{mixture}}}{dt} \quad (2)$$

The toluene flow rate (Q_{toluene} in $\text{g} \cdot \text{h}^{-1}$) was a controlled parameter in the experiment and toluene stripping ($Q_{\text{air}} C_{\text{gas}}$) was monitored over time. The overall toluene stripping in the gas output during the whole experiment was thus obtained by the cumulative addition of the stripping measured between two time intervals (Eq. 3).

$$Q_{\text{air}} C_{\text{gas}} = \sum_{t=0}^{\infty} Q_{\text{air}} \left(\frac{C_{\text{gas}}^{t+\Delta t} + C_{\text{gas}}^t}{2} \right) \quad (3)$$

In the same way, the accumulation term was deduced from Eq. (4), where C_{mixture} over time was calculated assuming that gas-liquid equilibrium was reached in the reactor (which can be reasonably assumed because the air residence time in the reactor was long, 13 min, and the toluene flow rate was very small compared to the volume of the mixture; Table 1).

$$V_{\text{mixture}} \frac{dC_{\text{mixture}}}{dt} = \sum_{t=0}^{\infty} \frac{V_{\text{mixture}}}{\Delta t} \left(\frac{C_{\text{mixture}}^{t+\Delta t} - C_{\text{mixture}}^t}{2} \right) \quad (4)$$

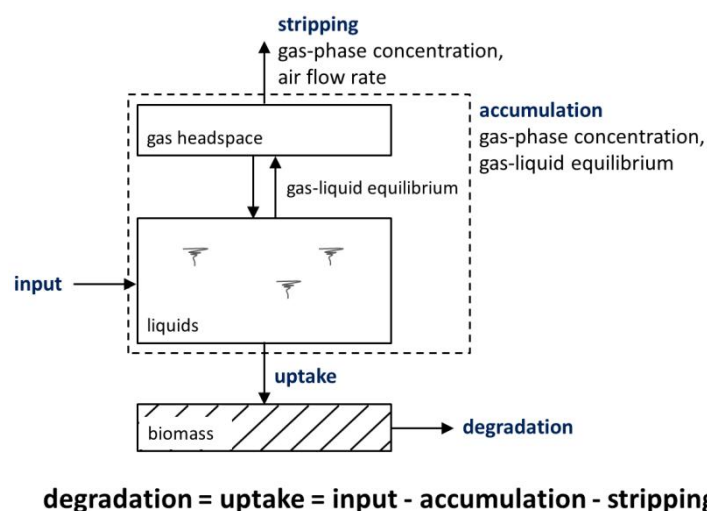


Figure 2 Calculation of the toluene degradation rate

3. Results and discussion

3.1. Sequential experiments

Sequential experiment was carried out in duplicate in order to assess the reproducibility of degradation rates and biomass measurements. After the first toluene injection, corresponding to the first day of experiment (day 1), a lag phase due to an acclimation period of about 20 h was observed. After the acclimation period, biomass activity started immediately after each toluene injection. An example of the time-course of the normalized amount of toluene in the liquid phase ($n_{(t)}/n_{(t=0)}$) recorded after the toluene injection ($t=0$) is shown in Fig. 3. The beginning and the end of the degradation can be directly determined from the simultaneous and dramatic changes in the O_2 and CO_2 concentration curves in the gas phase, as well as O_2 dissolved in the aqueous phase. It should be noted that the real CO_2 level reached during experiments cannot be known due to the saturation of the analyzer (plateau at 3 % CO_2). As observed in Fig. 3, the amount of oxygen dissolved in the liquid phase could not be considered a limiting factor. After each toluene injection, the biodegradation rate (accuracy ± 10 %) was determined using the curves reported in Fig. 3 and Eq. (1). It should be noted that for all experiments, the stripping of toluene in the air output was less than 10 % of the total amount of toluene injected into the mixture. The ten biodegradation rates determined during the sequential experiments are displayed in Fig. 4, which also shows the biomass concentration measured before each toluene addition. After the acclimation period, the biodegradation rate was roughly constant (until day 7) at around $1 \text{ g} \cdot \text{h}^{-1}$, i.e. $100 \text{ g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$. The amount of biomass increased daily. However, after day 7, the biodegradation rates dropped to 0.5 g h^{-1} (i.e. $50 \text{ g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$) while the biomass continued to increase. According to this figure, the biomass production can be correlated with the amount of degraded toluene. Taking into account the relative accuracy of the biomass concentration measurements due to the presence of PDMS, the yield was $0.055 \pm 0.011 \text{ biomass g} \cdot (\text{toluene g})^{-1}$, which corresponds to data reported in the literature [37]. The drop in the biodegradation rate is difficult to explain because (1) the dissolved oxygen concentration never became nil. As a result, the availability of O_2 also did not limit the degradation rate (a test carried out without aeration (not shown) highlighted that the concentration of dissolved O_2 could be nil, which limited biodegradation); (2) since the biomass increased continuously, the

amount of available biomass did not limit the biodegradation; (3) since the nutrients were added in excess at the beginning of each experiment, they could not be considered as a limiting factor. As the supply of toluene was sequential, the drop in the biodegradation rate could be due to an irregular availability of toluene, which should not be observed in the series of continuous experiments. Moreover, the occurrence of inhibitory metabolites due to toluene degradation could be contemplated and should be investigated in future works. Compared with the literature data, it appears that the biodegradation rates obtained are of the same order of magnitude as performances usually reported for conventional bioreactors for air treatment, from 10 to 70 $\text{g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ [6,38,39]. Studying toluene removal in laboratory-scale peat biofilters, Alvarez-Hornos et al. [40] reported an elimination capacity of 93 $\text{g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ at an Empty Bed Residence Time (EBRT) of 57 s. Moreover, it seems that higher performances could be reached using fungal strains *Paecilomyces variotti* and *Exophiala oligosperma*. Elimination capacity values as high as 164 $\text{g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ have been reported [41]. Clearly, although the selection of a pure culture for VOC degradation leads to better removal efficiency, the use of a mixed culture, such as activated sludge, for industrial applications is preferable, owing to its robustness.

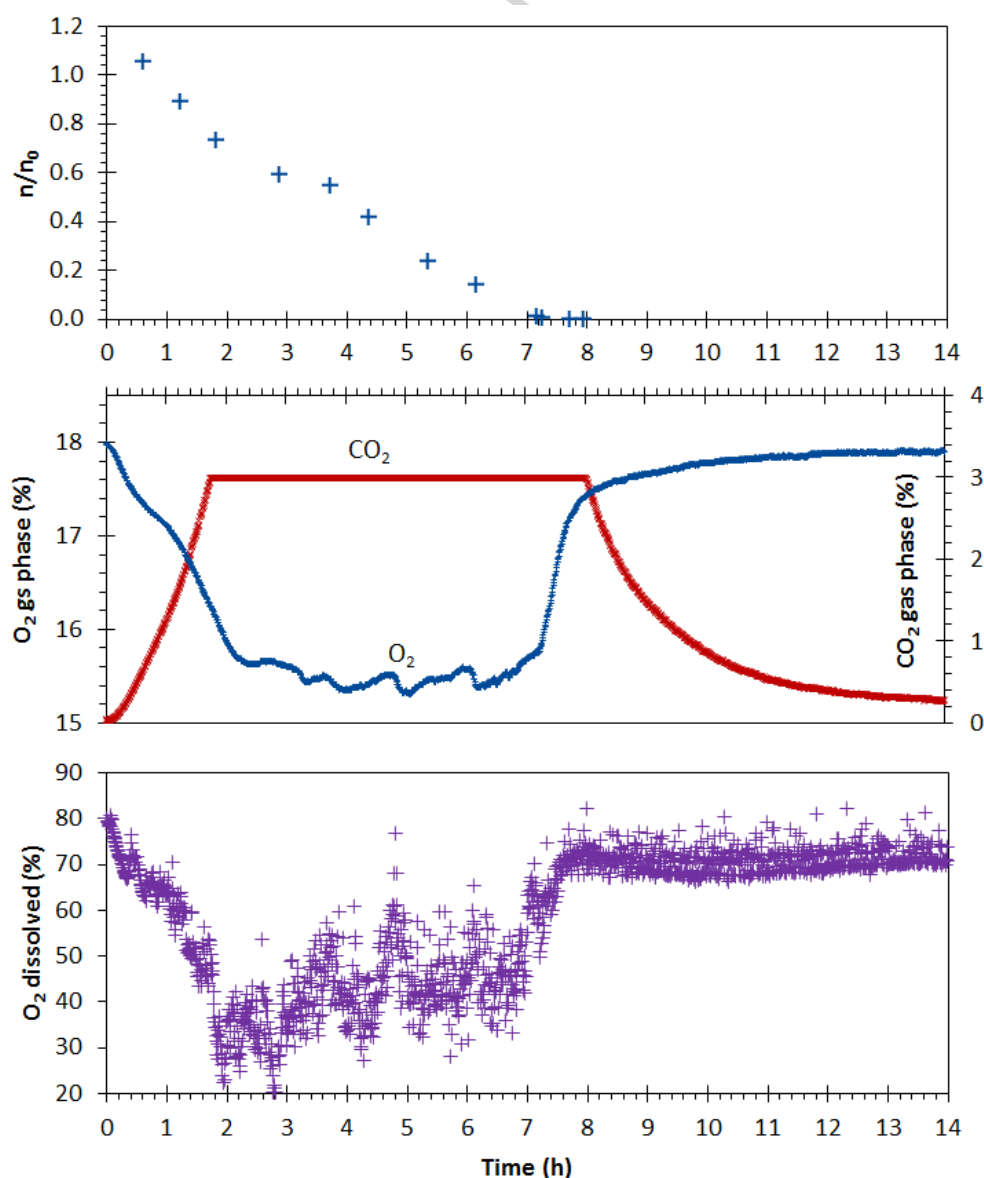


Figure 3 Sequential experiments: example of toluene biodegradation and parameters monitored in the gas phase and in the aqueous phase

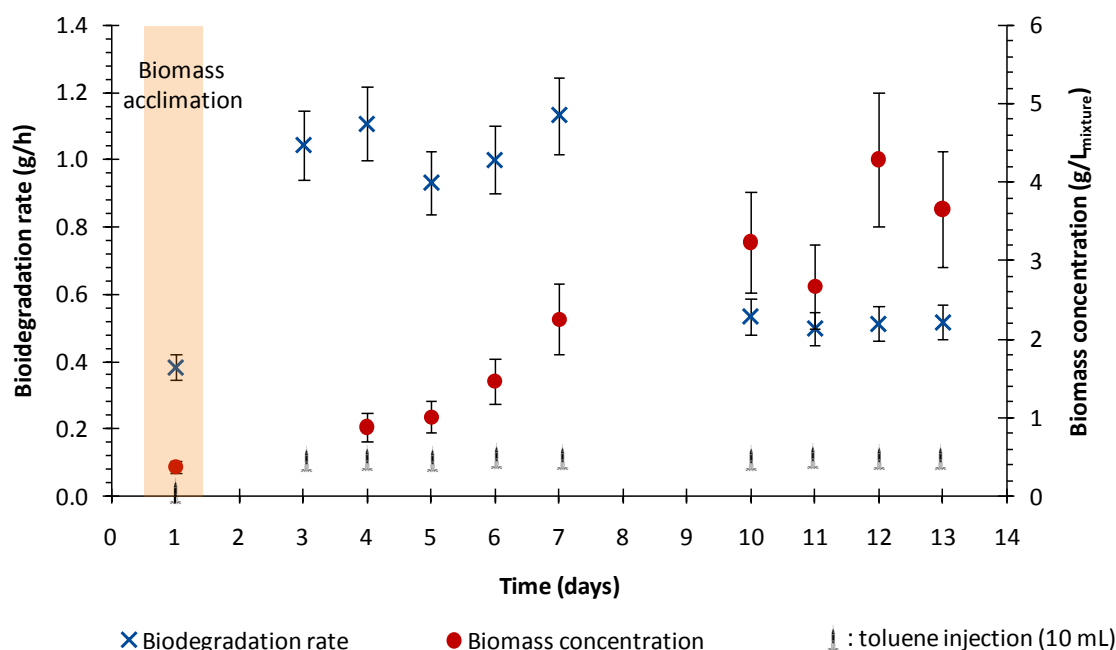


Figure 4 Biodegradation rates and biomass concentrations determined during sequential experiments

3.2. Continuous experiments

An example of the time-course of toluene changes during a continuous experiment is presented in Fig. 5. This figure shows, in the upper part, the mass balance of the amount of injected toluene between stripping, biodegradation and accumulation in the water/PDMS 50 mixture (Eq. 2) and, in the bottom part, the derivative curves corresponding to the stripping and biodegradation rates, respectively. As for sequential experiments, a lag phase was observed at the beginning of the experiment, due to the acclimation of the microorganisms to toluene. Hence, no degradation was observed during the first 20 h of culture and consequently toluene was predominantly accumulated in the liquid phase reactor. At the same time, a part of toluene was stripped from the reactor (between 5 and 10 %). The greatest stripping rate was monitored for the maximum amount of toluene accumulated in the liquid phase. After the initial lag phase, the degradation began and a peak in the toluene removal rate was observed after 25 h of treatment ($13.4 \text{ mmol} \cdot \text{h}^{-1}$). Once the toluene reserve was depleted, the microorganisms degraded toluene as soon as it was injected into the reactor, which is illustrated by the negligible residual gas-phase concentration from less than 45 h until the end of the experiment. Consequently, from this time, the toluene removal rate was equal to the injection rate (Fig. 5(b)). Oxygen concentrations in the liquid and gas phases were monitored during the course of experiments (insert in Fig. 5(b)). Concentrations in both phases followed the same trend. Roughly constant during the lag phase, the oxygen concentration dropped dramatically when toluene degradation started. The dramatic decrease in the oxygen concentration in both liquid and gas phases, down to values close to 50 % for the former and 15 % for the latter, corresponded to the high removal rate of toluene observed at the same time. It is noteworthy that even during this peak of consumption, oxygen

remained not limiting. During toluene degradation at a constant rate (after 50 h), oxygen concentrations stabilized at values close to 70 % and 16.5 % for dissolved and gas phase oxygen, respectively.

Toluene degradation was efficient ($RE = 100\%$) for toluene inputs ranging from 0.2 to 1.2 ml h^{-1} , (i.e. up to 11.3 $\text{mmol} \cdot \text{h}^{-1}$) which correspond to an elimination capacity of 104 $\text{g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$. It should be noted that this performance, which is consistent with the results recorded during the sequential experiments, corresponds to the degradation rate obtained at the end of the experiment, i.e. at steady-state (the biodegradation rate peak may be much higher as highlighted in Fig. 5). This result is two times higher than data reported by [27] using an airlift TPPB (water/PDMS 5, 90/10 v/v) to treat a mixture of BTEX (EC of 52 $\text{g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ corresponding to a toluene removal efficiency of 87.2 % for a loading rate of 60 $\text{g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$). Using the oxygen measurements in the gas phase between the beginning and end of the experiments, it was possible to calculate the amount of oxygen transferred during the degradation of toluene at steady-state for these operating conditions. Results are ranged from 5.0 to 6.5 moles of oxygen per mole of toluene. Taken into account the part of toluene stripped during the experiment, such results are in agreement with the expected value. Indeed, as described in Section 2.2., it is usually assumed that half of the organic compound is converted to cellular mass and half oxidized for energy [33]. With this assumption, the amount of oxygen required to biodegrade 1 mole of toluene is 6.5 moles (i.e. 4.5 moles for energy production and 2 moles for biomass production). The biomass production determined during the continuous experiments corresponded to that measured during the sequential experiments. Thus, at the end of the experiment displayed in Fig. 5, the biomass concentration was 1.5 dry mass $\text{g} \cdot (\text{mixture L})^{-1}$ (toluene injection: 0.4 $\text{ml} \cdot \text{h}^{-1}$), and values of 4.7 dry mass $\text{g} \cdot (\text{mixture L})^{-1}$ were recorded for a toluene injection of 1.2 $\text{ml} \cdot \text{h}^{-1}$. The trend of a linear increase in biomass concentration with elimination capacity is in agreement with the result reported by Littlejohns and Daugulis [27]. Using an airlift TPPB to treat a mixture of BTEX (Benzene, Toluene, Ethylbenzene, *o*-Xylene), these authors obtained a linear correlation between the average EC and biomass concentration. However, this result was obtained using silicone rubber beads (10 % v/v) as the non-aqueous phase.

Conclusions

Experiments were carried out in a semi-continuous stirred tank reactor to determine the ability of a mixture of water/silicone oil PDMS 50 (75/25 v/v) to degrade toluene. The performances of biodegradation obtained from sequential and continuous experiments, up to 104 $\text{g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ ($RE = 100\%$), are thus of primary importance in designing the stirred tank reactor for large-scale applications. Based on this laboratory result, a pilot device coupling the absorption step of toluene by PDMS in a separate column with the biodegradation step in a TPPB can now be designed and tested on an industrial site to study the biodegradation performances on a real effluent loaded with toluene. The next work is to confirm over a long period the ability of a TPPB to degrade toluene and to study the impact of the possible accumulation of inhibitory metabolites due to biomass activity. The biodegradation performances will be studied for sequential and continuous operating conditions encountered in industrial companies. The transient-state conditions and shock-loads will be also investigated.

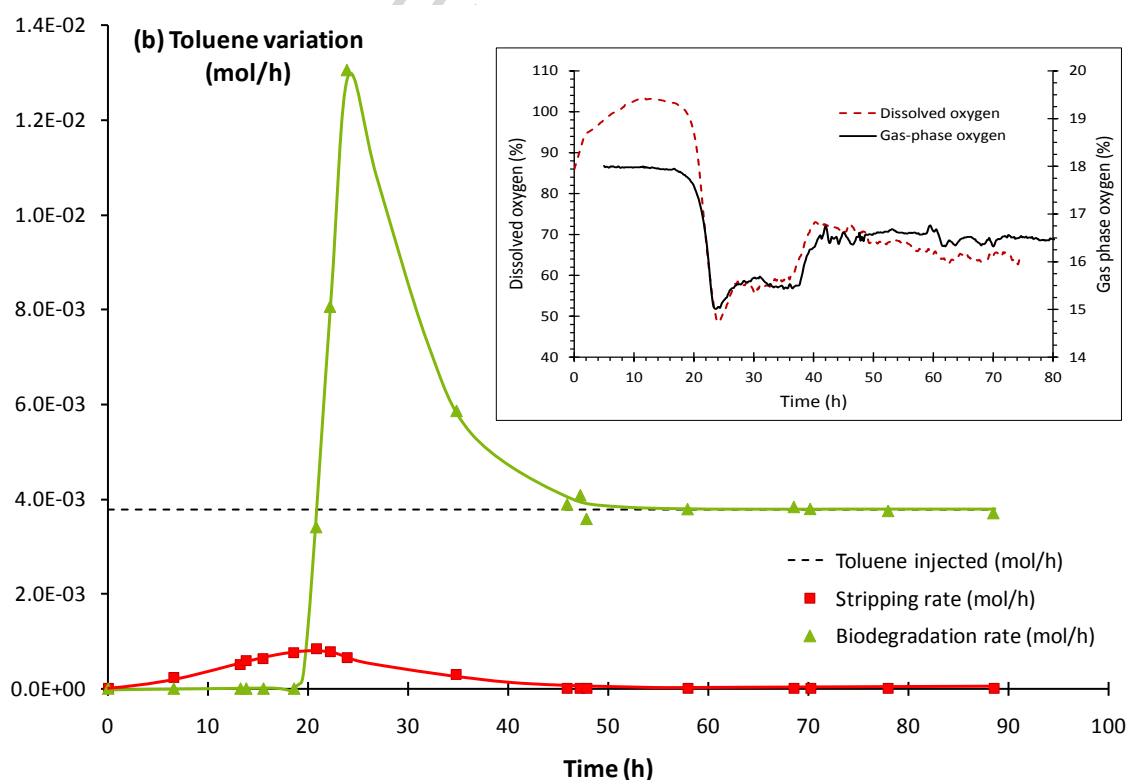
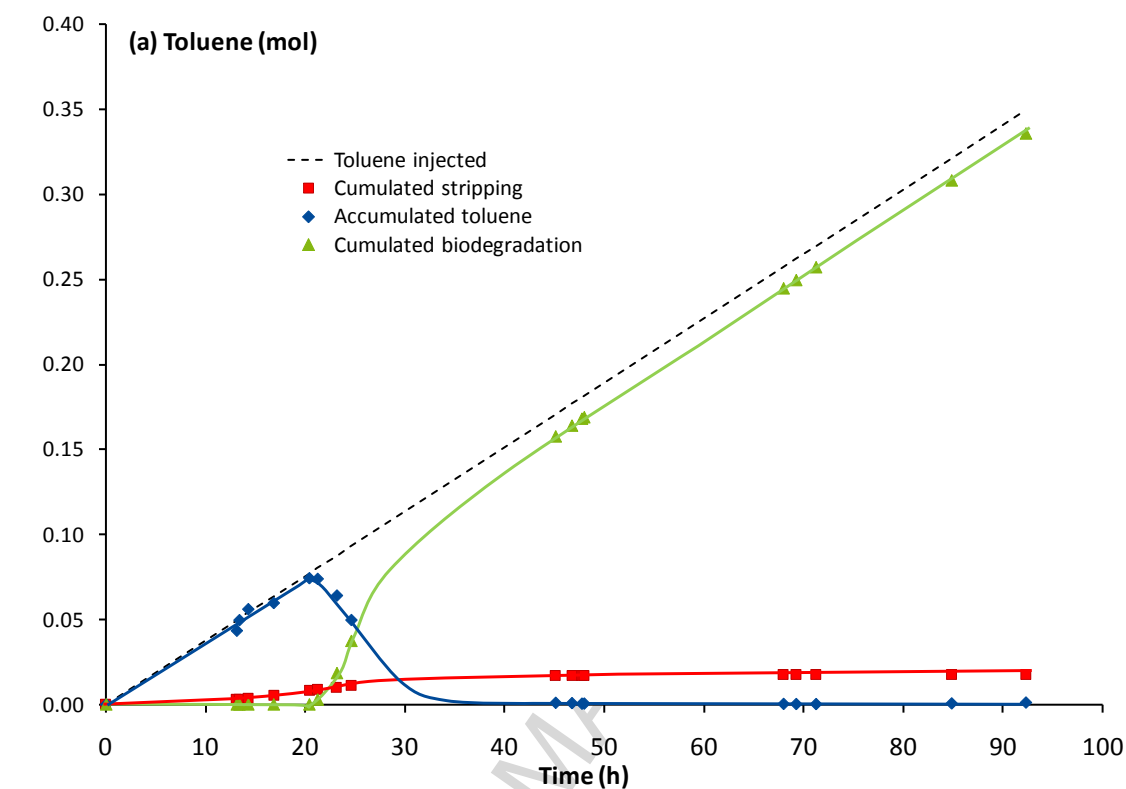


Figure 5 Continuous experiments: example of the determination of toluene biodegradation (toluene injection: $0.4 \text{ ml} \cdot \text{h}^{-1}$, i.e. $3.8 \text{ mmol} \cdot \text{h}^{-1}$)

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To whom it may concern,

I certify that I have read and corrected the English of the article:

Toluene Biodegradation in a Two-Phase Partitioning Bioreactor

on behalf of Eric Dumont of L'UNAM Université, École des Mines de Nantes.

I am a native English speaker with a degree in Biochemistry and many years of experience in editing scientific texts.

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