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To cite this version:

I. Bechohra, J. B. Le Menn, A. Couvert, A. Amrane. Activated sludge acclimation for toluene and DEHP degradation in a two-phase partitioning bioreactor. International Journal of Environmental Science and Technology, 2016, 13 (8), pp.1883–1890. 10.1007/s13762-016-1019-y . hal-01357405

HAL Id: hal-01357405

https://univ-rennes.hal.science/hal-01357405v1

Submitted on 2 Dec 2016

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Activated Sludge Acclimation for toluene and DEHP degradation in a Two-Phase Partitioning Bioreactor

Biodegradation of volatile organic compounds by activated sludge

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Abstract

The effect of activated sludge acclimation on the biodegradation of toluene in the presence of a biodegradable Non-Aqueous Phase Liquid (NAPL), di (2-ethylhexyl) phthalate (DEHP), in a two-phase partitioning bioreactor was characterized. The influence of the presence of DEHP, at a ratio of 0.1 % (volume ratio), and of the acclimation of activated sludge on the biodegradation of hydrophobic VOC was studied. Activated sludge was acclimated to both toluene and DEHP simultaneously. Using acclimated cells, 73 % and 96 % improvement of the mean biodegradation rates were recorded for toluene and the organic solvent (DEHP) respectively, if compared to the values recorded in the absence of acclimation, during tests performed in Erlenmeyer flasks. Degradation rates were further improved by the use of acclimated activated sludge (AS) in a reactor with a large head space; degradation yields for toluene and DEHP were above 99 % and 89 % respectively.

Keywords: Toluene; DEHP; activated sludge; biodegradation; acclimation.

Introduction

The removal of volatile organic compounds (VOCs) from contaminated airstreams has become a major public concern (Malhautier et al. 2014). They are produced by a variety of industries and their emissions are facing increasingly stringent environmental regulations because they affect public health and welfare owing to their toxicity, potential carcinogenicity and stability (Spigno et al. 2003). Biological techniques have attracted considerable interest in recent years because they offer a number of advantages when compared to other traditional air pollution technologies, such as combustion, oxidation, etc (Smith 1991).

However, despite their inherent advantages, the performance of biological gas treatment processes is often challenged by the hydrophobicity of some specific VOCs (such as toluene, benzene), which limits pollutant transfer from the gaseous phase to the aqueous phase containing the microorganisms responsible of pollutants biodegradation (Collins and Daugulis 1999; Arriaga et al. 2006; Hernández et al. 2010; Muñoz et al. 2012). The process limitation in conventional biotechnologies arises from microbial inhibition or O_2 limitation during the treatment of high concentrations of water soluble or moderately soluble VOCs, especially during episodes of load surges (Collins and Daugulis 1999; Muñoz et al. 2015).

Two-phase partitioning bioreactors (TPPBs), which are based on the addition to the biological process of solid or liquid non-aqueous phases (NAPL), can enhance the transfer of hydrophobic VOCs, as a result of the high affinity of the organic phase for these latter, as well as that of O_2. Therefore, this technology has supported unprecedentedly high elimination capacities (EC) and at the same time decreases target compounds toxicity towards microorganisms by lowering their concentration in the aqueous phase (Van Groenestijn and Lake 1999; Tomei et al. 2010). For instance, some authors reported, for α-pinene, elimination capacities at steady state 10 to 12 times higher than those recorded in a similar system without any liquid NAPL (Hernández et al. 2011).

To avoid costly NAPL, such as silicone oils and ionic liquids, which showed to be relevant for implementation in a TPPB (Darracq et al. 2010a; Quijano et al. 2011b; Dumont et al. 2011; Quijano et al. 2011a), but should be recycled owing to their cost, another alternative is to consider an industrial waste containing an organic phase (Chikh et al. 2011; Béchohra et al. 2014). A biodegradable solvent (hexadecane) was used for this purpose as a model solvent. In this case, the solvent should be biodegradable leading to its removal, concomitantly with the target VOC.

This work deals with the implementation of DEHP (Di (2-ethylhexyl) phthalate) in a TPPB, found to be efficient for both the absorption capacity step and at the biodegradation level, since it is biocompatible, non-soluble in

water and biodegradable (Madsen et al. 1999; Feng et al. 2002; Chao and Cheng 2007; Béchohra et al. 2015). The hydrophobic targeted VOC is toluene, often encountered in gaseous effluents of pulp and paper factories, composting facilities, food industries or other industries (Rappert and Müller 2005; Jin et al. 2007; Darracq et al. 2010b).

The impact of the ratio DEHP/water on the biodegradation performances was previously examined, showing an optimum of 0.1 % (volume ratio) for simultaneous degradation of DEHP and toluene (Béchohra et al. 2015). Indeed, on the one hand, the NAPL quantity must be sufficient to allow a good VOC mass transfer from the organic phase to the aqueous phase, and on the other hand, a too high NAPL amount can be toxic for microorganisms.

The objective of this paper was to characterize the effect of activated sludge acclimation on the biodegradation of toluene and DEHP in the optimal conditions, namely a DEHP to water volume ratio of 0.1% in the TPPB. Kinetics of the VOC removal was also examined. Considering that both NAPL and VOC can be toxic for microorganisms, preliminary tests were carried out regarding the interest of an acclimation to both compounds; then, the performances reached with activated sludge acclimated to each VOC in the presence of DEHP were compared with those obtained with non-acclimated activated sludge.

Materials and methods

VOC and Solvent

The selected VOC was toluene from Carbo Erba reagenti and the solvent used was DEHP (MW = 390.60 $g.mol^{-1}$, d = 0.970, $T_{boiling}$ = -50 °C) from Acros organic.

Microorganisms

The biomass used in this work was activated sludge (AS) from Beaurade, the municipal wastewater treatment plant of Rennes (France) and were maintained in a lab-reactor (Chikh et al. 2011; Béchohra et al. 2014).

DEHP biodegradability

Biodegradability was measured using BOD OxyTop (WTW, Alès, France). AS from a wastewater treatment plant was used to inoculate samples, which consisted in various volume fractions of DEHP in water, 0.1, 0.5, 2 and 5%, a blank and the control solution. The initial microbial concentration of dry weight was 0.05 $g.L^{-1}$. The following mineral basis was used for all experiments ($g.L^{-1}$): $MgSO_4 \cdot 7H_2O$, 22.5; $CaCl_2$, 27.5; $FeCl_3$, 0.15; NH_4Cl, 2.0; Na_2HPO_4, 6.80; KH_2PO_4, 2.80 and N-allylthiourea, 10 $mg.L^{-1}$. The BOD_5 value was initially estimated based on the COD value experimentally measured by means of a Nanocolor Test (Macherey-Nagel, Düren, Germany), according to BOD_5 = COD/1.46. The range of expected BOD_5 measurement was then deduced and hence led to the volumes of sample, of AS solution.

A similar protocol was applied for the control flask except that except that the content has been replaced by a solution of easily biodegradable compounds, namely glutamic acid (150 $mg.L^{-1}$) and glucose (150 $mg.L^{-1}$). Before use, NaOH was added to achieve neutral pH (7.0±0.2). Finally, a similar protocol was also considered for the blank solution, for which the sample was replaced by water to have a negligible BOD_5 value (Darracq et al. 2010a; Ferrag-Siagh et al. 2013).

Batch and reactor cultures

Experiments were conducted in 250 mL Erlenmeyer flasks closed with cellulosic caps for the low concentration of toluene (4.3 $mg.L^{-1}$) to allow air exchange during the first set of experiments. For the high concentration of toluene (106 $mg.L^{-1}$), glass caps were used. Erlenmeyer are equipped with two sampling points sealed with Teflon septum allowing needle introduction. To sample the gaseous phase, a micro syringe with a capacity of 500 µL was used and the sample was injected directly in the gaseous chromatography (GC); aqueous samples could be stored in closed vials for further analysis of by-products. The Erlenmeyer headspace was 50 mL. For each experiment, a given number of identical 250 mL Erlenmeyer flasks were considered. Initially, concentrations of 4.3 and 106 g of toluene per liter of DEHP at 0.1% volume fraction were considered. Each series of experiments consisted of 13 flasks containing 0.5 $g.L^{-1}$ of activated sludge and nutrients. A blank test containing the considered amount of DEHP was also carried out to determine the fraction of VOC lost by possible leaks or adsorption on the cellulosic stoppers; its composition was the same as the other flasks except for nutrients and biomass. Stirring was set at 300 rpm and flasks were placed in a thermostated oven (T = 25°C). Because of the biodegradability and the negligible solubility of DEHP in the aqueous phase, it was not possible to perform a homogenous sampling of the two-phase system and hence a sacrificial method was considered, at pre-determined time intervals, duplicate Erlenmeyers were taken for analysis. Gaseous samples were analyzed to

quantify the remaining toluene quantity (Béchohra et al. 2015).

Experiments in a semi-batch reactor (closed for the liquid phase but open for the gas phase) of 2 liters were performed with the same organic solvent (0.1%) and different toluene concentrations in the DEHP (43. 106 and 212 g of toluene / L of DEHP). Stirring was set at 300 rpm in a thermostated bath (T = 25 °C). A blank test without AS was carried out for each concentration to quantify the leakage toluene.

The amounts of toluene in the gas phase, pH and the suspended solid quantity were followed. The sampling of the aqueous phase was also carried out to monitor by-products; the formed CO_2 was also monitored and quantified by a Infrared Fourier Transform (FTIR) brand Environment SA spectrophotometer (Cosma reference Beryl 100).

Acclimation of activated sludge to toluene and DEHP

Activated sludge acclimation to VOC and NAPL was performed as follows: a washed solution of AS was put into a closed 1 L reactor; a Trinci solution at 50 $ml.L^{-1}$ (Trinci 1969), nitrogen in the form of NH_4Cl and phosphorus in the form of KH_2PO_4 and NaH_2PO_4 were added. Increasing amounts of toluene and organic phase were added, starting with 20 µL of toluene and 50 µL of DEHP the first week and increasing 20 µL of toluene and 50 µL of organic phase each week. After each toluene exhaustion, air bubbling was carried out, pH (7 ± 0.2). Every week, the culture medium was supplied with 10 mL of Trinci solution, 190 mL of water, 5 mL in nitrogen and 2.5 mL of phosphate after a bleeding of 200 mL of the supernatant after decantation.

Analytical Methods

The toluene concentration in the gas phase was measured by gas chromatography (GC) coupled with a flame ionization detector from Thermo scientific (California, United States). Metabolites formed in the aqueous phase were identified by gas chromatography coupled with mass spectrometry (MS) with headspace (HS) from Perkin Elmer (California, United States). For the quantification of DEHP degradation, an extraction by hexane (25% volume ratio) coupled to 10 min ultrasonication was performed. The extract was then analyzed by gas chromatography coupled with a flame ionization detector from Perkin Elmer (California, United States). The analytical conditions are reported in Table 1.

The total amount of toluene m_t was then deduced from the mass balance (Equation 1):

$$m_t = m_{tg} + m_{te} \qquad (1)$$

m_{tg}, m_{te} were the toluene mass in the gaseous and emulsion (DEHP in water) phase at a given time t; m_{tL}, the toluene leakage, was deduced from the blank test between the initial time and a given time t.

$$m_{tL} = (m_{0gb} + m_{0eb}) - (m_{tgb} + m_{tb}) \qquad (2)$$

m_{0gb}, m_{0eb} were the toluene mass of the blank test in the gaseous phase and the emulsion at initial time m_{tgb} and m_{teb} were the toluene mass of the blank test at a given time t in the gaseous phase and the emulsion.

The concentration in the emulsion at a given time t and in the blank test can be then deduced from the partition coefficient H for different volume fractions (Béchohra et al. 2015) and from the toluene concentration in the gaseous phase at the given time t (Eq. 3):

$$C_e = C_G/H \qquad (3)$$

The mineralization rate was determined for reactor tests, with and without acclimation; it is defined as the ratio of total carbon moles of CO_2 to the number of moles of carbon consumed or initially introduced with the carbon substrates (Solano-Serena et al. 2001), as represented by equation (4):

$$\%\, mineralization = {nC_{CO2}}/{nC_X} \qquad (4)$$

With nCO_2, the total amount of CO_2-carbon formed and nCx the total amount of carbon initially introduced.

The biomass growth was quantified by means of turbidimetric measurements at 600 nm which was related to dry matter (DM) through a calibration curve.

Results and discussion

DEHP biodegradability

Figure 1 shows DEHP biochemical oxygen demand for the four volume ratios DEHP / water (5 %, 2 %, 0.5 % and 0.1 %) with a blank test without carbon source and a control. As expected, a very significant increase of the BOD_5 value of the control, a solution of glucose and glutamic acid, can be noted, showing the activity of the used activated sludge; while the BOD_5 value of the blank, corresponding to the endogenous respiration of the microorganisms remained low throughout the 5 days experiment (5 mg $O_2.L^{-1}$ – Table 2).

An increase over time was observed for the different volume ratios of DEHP; 50 mg $O_2.L^{-1}$ was obtained for 0.1 % DEHP and 40 $mgO_2.L^{-1}$ for the other ratios. This leads to the conclusion that DEHP is used by sludge as carbon source and therefore it is biodegradable. Several authors have studied the biodegradability of DEHP by bacterial strains (Feng et al. 2002; Chen et al. 2007), but also by mixed crop (Chao and Cheng 2007) and activated sludge (Wang et al. 1996).

BOD_5 values were obtained after subtracting the blank values. All values regarding biodegradability are collected in Table 2, showing that after subtracting the endogenous respiration all ratios BOD_5 on COD values were above 0.4, indicating that DEHP was a biodegradable compound.

Biodegradation kinetics

Erlenmeyer experiments

Biodegradation tests were carried out with acclimated and non-acclimated activated sludge; time-courses of toluene degradation are shown in Figure 2. Significant losses due to toluene leakages were observed, leading to only 9-10 % degraded, while the rest corresponded to leakages. As previously observed, the use of cellulose caps led to important losses, especially for low DEHP in water ratios (Béchohra et al. 2015). It can also be noticed that after acclimation, losses were somewhat reduced, since the acclimated biomass is adapted to the substrate which is therefore more rapidly consumed and then the stripping is limited (Mozo et al. 2012). To reduced toluene leakages, glass caps were previously shown to be relevant, since losses were reduced by 29 % (Béchohra et al. 2015).

DEHP biodegradation was also examined, showing for both AS a decrease of the DEHP amount in the beginning of the cultures, before reaching a constant value between the 1st and the 4th day for non acclimated AS and between the 1st and the 2nd day for acclimated AS (Figure 3). This stationary state may be due to the presence of by-products detected by GC/MS, including 2-ethylhexanol and 3-méthyl, 3-heptanol. According to the literature, by-products are described as being more toxic than the original molecule (Horn et al. 2004). Beyond this phase, a second step of DEHP degradation was observed, certainly due to acclimation of activated sludge to by-products; until reaching 92.4 % and 98.0% decrease after seven days for non-acclimated and acclimated AS. Some authors have also observed a complete DEHP degradation for an initial solvent concentration of 0.75 $g.L^{-1}$ (corresponding to 0.08 % DEHP/water volume ratio) in only forty hours, but using a pure *Gordonia* sp. (Sarkar et al. 2013).

Toluene concentration 106 mg.L^{-1} of emulsion

A concentration of 106 g of toluene per liter of DEHP, corresponding to 106 mg of toluene per liter of emulsion, was previously tested in order to study the microorganisms' behavior with a such high toluene concentration (Béchohra et al. 2015), but acclimated sludge was not considered; tests were therefore performed considering non-acclimated and AS acclimated to toluene and DEHP.

A lag phase of about 48 hours was observed in the case of non-acclimated activated sludge (Figure 4); then a decrease of the toluene amount until its complete removal (60 % of biodegradation), taking the losses into account. On the other hand, in the case of acclimated sludge no lag phase was observed and total toluene consumption was observed after 24 hours of culture, which is confirmed by its absence in the gas phase, and the final degradation yield was 72.7 %; the rest corresponded to the losses.

With non-acclimated AS, there was a significant decrease in the amount of DEHP over time until a final mass of 0.03 g, namely 87 % biodegradation at the end of the experiment (Figure 5). For acclimated sludge, there was an increase of both the degradation rate and the degradation yield, with an almost total removal at the end of culture, 96 %. These results are in agreement with microbial growth, which was higher in the case of acclimated activated sludge (not shown).

Reactor experiments

Initial toluene concentration

Three toluene initial concentrations, 43, 106, 212 g of toluene per liter of DEHP were tested in the reactor with a liquid volume of 2 liters and a head-space volume of about 1 L. The toluene concentration monitoring is shown in Figure 6. In order to avoid toluene stripping due to air sparging, bubbling with air was carried out at the beginning of the experiment before closing the reactor and introducing toluene; therefore, microorganisms should use the oxygen trapped in the headspace during the biodegradation process. A decrease in the toluene mass was noticed in the gas phase for the high toluene concentration (212 mg.L^{-1} of emulsion or 212 g of toluene per liter of DEHP) until its complete removal after 7 days of culture (degradation rate of 7.41 mg.L^{-1}.h^{-1}); toluene degradation yield was 86 % at the end of the experiment, taking the leakages into account.

For the lowest toluene concentration (43 $mg.L^{-1}$ of emulsion), there was a total consumption of toluene (99 %) after 33 hours with a degradation rate of 4.90 $mg.L^{-1}.h^{-1}$.(Figure 6). It can be noted that the blank test (test without activated sludge) showed negligible leaks for this toluene concentration.

Figure 6 also shows that the toluene amount decreased until its complete removal after a period of 55 hours. Furthermore, after two days of culture, a toluene degradation by-product was detected in the aqueous phase, methyl phenol, confirming some results reported in the literature (Johnson and Olsen 1997). The mean toluene degradation rate was 2.92 $mg.L^{-1}.h^{-1}$ with a final yield of 86 %, namely significantly higher than the rate recorded in Erlenmeyer flasks, namely 0.72 $mg.L^{-1}.h^{-1}$. A blank test was also performed to quantify leaks and take them into account in the mass balance; they were 13%, namely significantly lower than those observed in Erlenmeyer flasks (40 %).

DEHP Monitoring could not be done, since DEHP sampling was not easy in the reactor; all the content of the reactor would be needed for its quantification. Because of the negligible solubility of DEHP in the aqueous phase, it was not possible to perform a homogeneous sampling of the two-phase system. The dry matter concentration was monitored daily by sampling the aqueous phase; a bacterial growth was then observed (not shown).

DEHP degradation yields for the three toluene concentrations tested are reported in Table 3, showing that the highest degradation yield was obtained for the toluene concentration of 106 $mg.L^{-1}$.

Successive batches

Even in the presence of air bubbling before toluene addition, an oxygen limitation was observed. The most appropriate would be to have a continuous air inlet, but it was not possible owing to toluene leaks by stripping; air was therefore bubbled into the liquid phase after total toluene consumption and before its reinjection. The corresponding test was performed and the results are shown in Figure 7. In the case of non-acclimated AS, the rate of degradation increased after addition of toluene, 7.14 $mg.L^{-1}.h^{-1}$ against 3.67 mg $L^{-1}.h^{-1}$ initially, showing an adaptation of the microorganisms to the pollutant.

For acclimated AS, only 24 hours were needed for microorganisms to completely degrade the toluene present and the mean degradation rate was 5.21 $mg.L^{-1}.h^{-1}$, and almost twice (9.09 $mg.L^{-1}.h^{-1}$) after toluene reinjection. A total of five toluene reinjections were operated with air bubbling prior to inject toluene, without any significant decrease in the toluene degradation rate.

The DEHP degradation rate after 7 days (end of the run) was 75 % for non-acclimated AS, which was found to be more important than the yield achieved without air bubbling before toluene reinjection (60 %) (results not shown). Degradation by-products were detected, namely 2-ethylhexanol and 3-methyl-3-heptanol. In the case of acclimated AS, the DEHP degradation yield increased until 89 % at the end of the run (7 days). DEHP metabolites were detected at the end of culture, 2-ethyl hexanal, 3-methyl-3-heptanol and 2-ethylhexanol as it was reported by (Horn et al. 2004), but these latter did not seem to negatively impact toluene or DEHP consumptions by the microorganisms.

Mineralization yields obtained after the degradation of toluene and DEHP by non-acclimated and acclimated activated sludge were 32.3 % and 51.9 % respectively. As expected, it was significantly higher in the case of acclimated activated sludge. Leaks are low and non-mineralized amount corresponded to degradation by-products such as those identified by GC/MS.

Conclusion

Biodegradation enhancement after microbial acclimation and in the presence of a biodegradable non-aqueous phase liquid (DEHP) was studied through their impact on the biodegradation yields and rates of carbon sources consumption. Degradation experiments of DEHP and VOCs were performed in Erlenmeyer flasks and in a semi-continuous reactor for a volume ratio of DEHP to water of 0.1 %. The experiments realized in batch revealed significant degradation for an optimal toluene concentration of 106 $mg.L^{-1}$ of emulsion or 106 $g.L^{-1}$ of DEHP: 73 and 60 % for toluene for acclimated and non-acclimated AS respectively, 96 and 73 % for DEHP for acclimated and non-acclimated AS respectively. These positive results were confirmed during experiments carried out in a reactor involving acclimated cells: degradation yields were more than 99 % and 89 % for toluene and DEHP, respectively.

Acknowledgements

The technical support of this research was provided by the National School of Chemistry of Rennes. Appreciation is extended to Mrs Marguerite Lemasle, for her help during the analysis of samples by GC/MS.

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The authors declare that they have no conflict of interest.

Table1 Analytical conditions implemented to determine the amount of VOC and organic solvent

Analyte	**Apparatus**	**Column**	**Detector**	**T_{inj} (°C)**	**T_{oven} (°C)**	**T_{det} (°C)**	**Carrier gas**
Toluene in the gas phase	Thermo focus GC	CP-FFAP CB; 25m*0.32mm	FID/Split	200	100	250	N_2 : 3.7 $mL.min^{-1}$
Metabolites in the aqueous phase	Perkin Elmer Clarus 500 GC/MS	CP-FFAP CB; 30m*0.15 mm	MS/HS	80	40-200		He : 1 $mL.min^{-1}$
DEHP in hexane	Perkin Elmer	CP-FFAP CB; 25m*0.32mm	FID/Split	200	160-260	250	N_2 : 1 $mL.min^{-1}$

Table 2 COD and BOD_5 for different volume ratios in DEHP in water (mg $O_2.L^{-1}$)

	0.1%	**0.5%**	**2%**	**5%**
COD $_{sample}$	64.5 ± 0.5	68 ± 1	70.5 ± 0.5	72.5 ± 7
BOD_5 $_{sample}$	50 ± 5	40 ± 5	40 ± 5	40 ± 5
BOD_5 $_{Blank}$	5	5	5	5
BOD_5 $_{without\ endogenous\ respiration}$	45 ± 5	35 ± 5	35 ± 5	35 ± 5
BOD_5/COD	0.70	0.51	0.49	0.48

Table 3 DEHP degradation rate for different initial toluene concentrations

Toluene ($mg.L^{-1}$)	**Degradation yields (%)**
212	79
106	95
43	72

Fig.1 Biological oxygen demand after 5 days (BOD_5) for various ratios of DEHP in water

■ day 1 ■ day 2 ■ day 3 ■ day 4 ■ day 5

Fig.2 Time-courses of toluene total amount during biodegradation by non-acclimated and acclimated activated sludge in batch culture. 4.3 g toluene.L^{-1} DEHP, 0.1 % DEHP (volumic ratio), pH = 7, T = 25°C, stirring speed = 300 rpm

□ Non-acclimated AS ◆ Acclimated AS

Fig.3 Time-course of DEHP amount during biodegradation by non-acclimated and acclimated AS in batch culture. 4.3 g toluene.L^{-1} DEHP, 0.1 % DEHP in water, pH = 7, T = 25°C, stirring speed = 300 rpm

□ Non-acclimated AS ◆ Acclimated AS

Fig.4 Time-course of toluene total amount during biodegradation by non-acclimated and acclimated AS in batch culture. 106 g toluene.L^{-1} DEHP, 0.1 % DEHP in water, pH = 7, T = 25°C, stirring speed = 300 rpm

□ Non-acclimated AS ◆ Acclimated AS

Fig.5 Time-course of DEHP amount during biodegradation by acclimated and non-acclimated AS in batch culture 106 g toluene.L^{-1} DEHP, 0.1 % DEHP in water, pH = 7, T = 25°C, stirring speed = 300 rpm

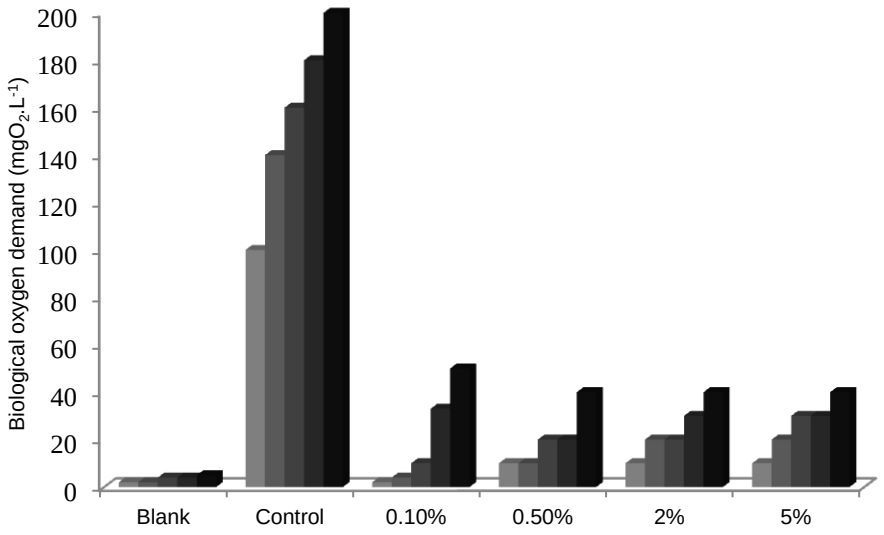
□ Non-acclimated AS ◆ Acclimated AS

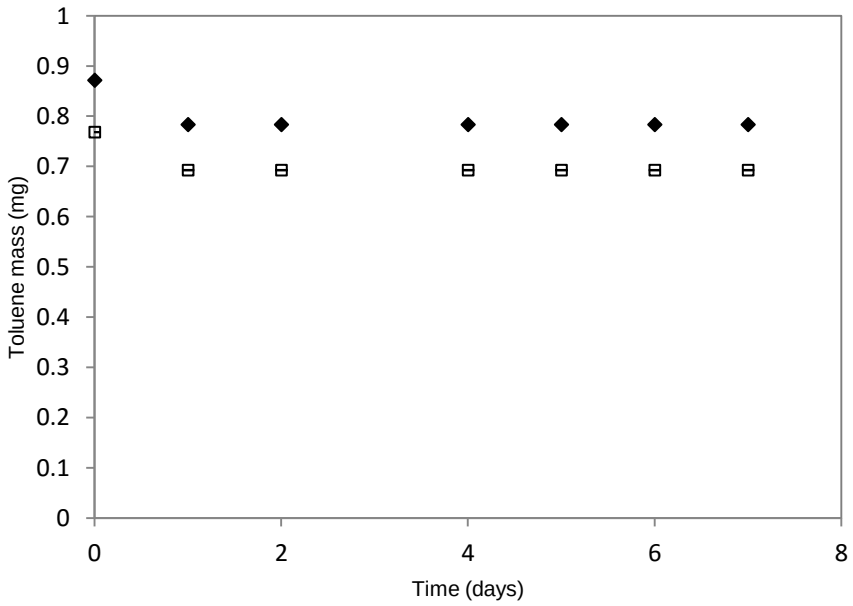
Fig.6 Time-course of the total toluene amount during biodegradation by activated sludge in batch culture for various toluene concentrations, 0.1 % DEHP in water, pH = 7, T = 25°C, stirring speed = 300 rpm

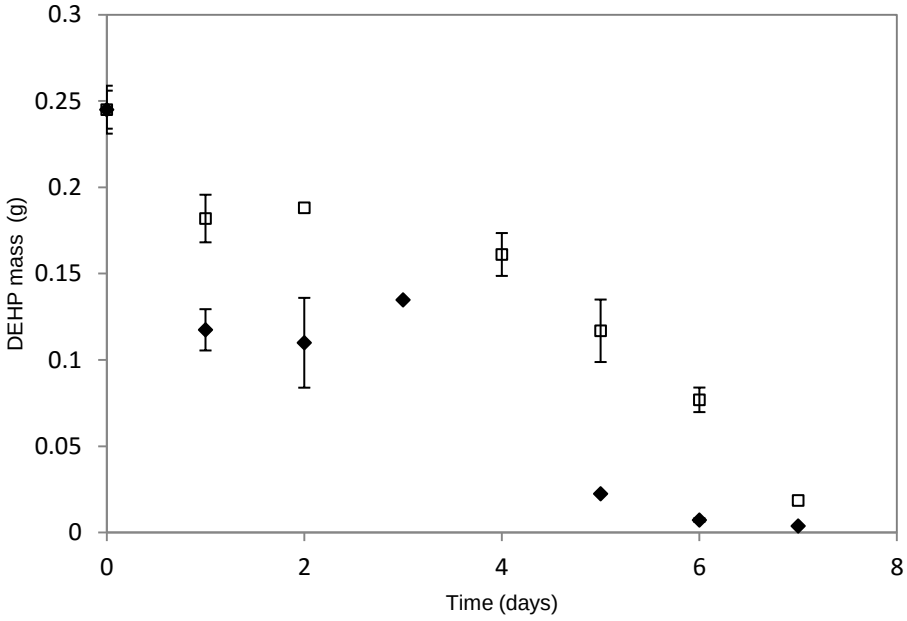
◆ 212 mg.L^{-1} □ 106 mg.L^{-1} △ 43 mg.L^{-1}

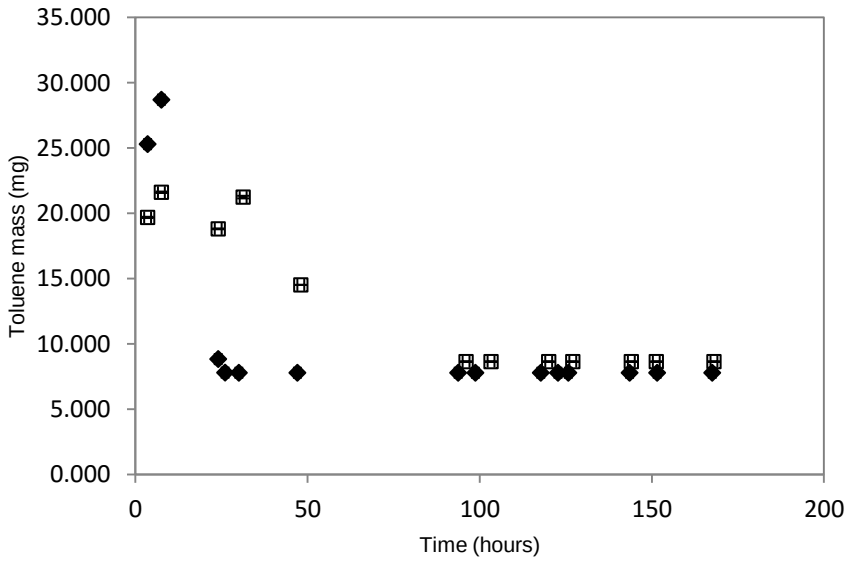
Fig.7 Time-course of toluene total amount during biodegradation by non-acclimated and acclimated AS in batch culture. 4.3 g toluene.L^{-1} DEHP, 0.1 % DEHP in water, pH = 7, T = 25°C, stirring speed = 300 rpm

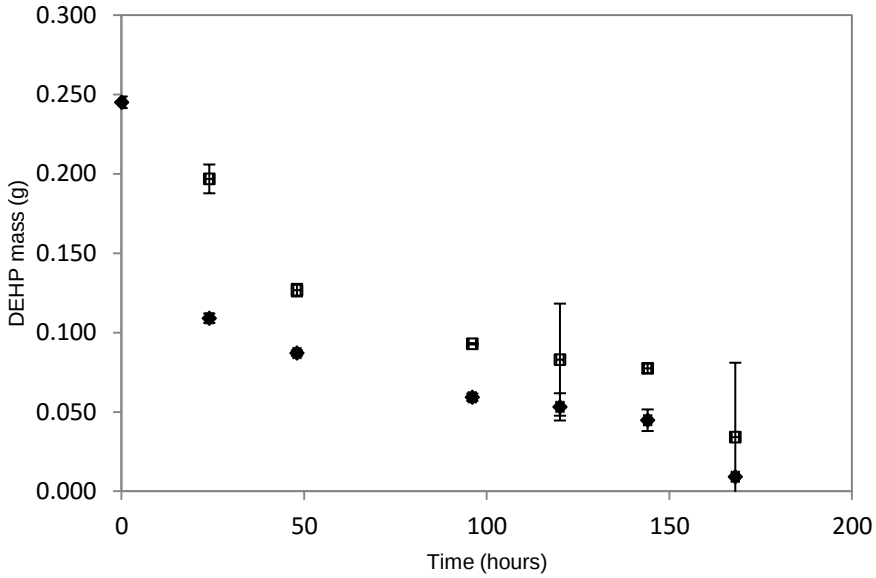
□ Non-acclimated AS ◆ Acclimated AS

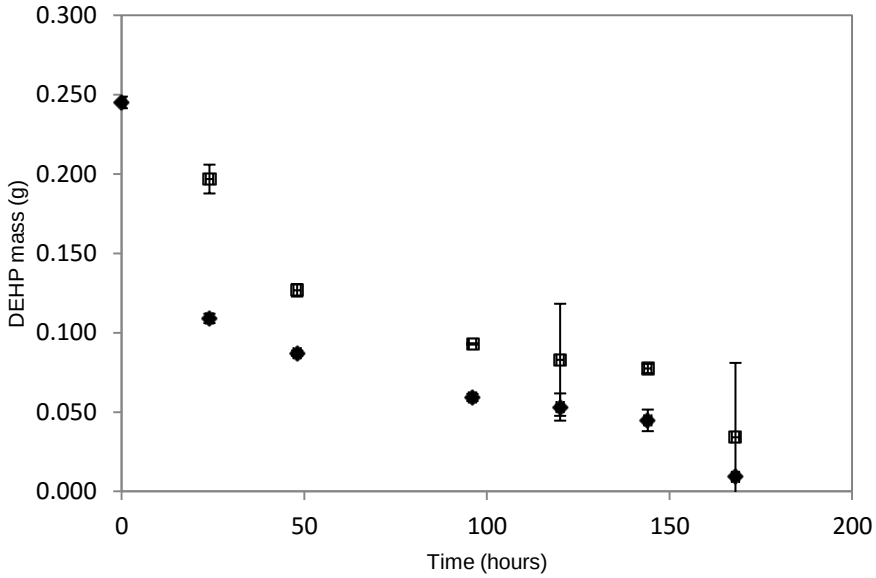

Biological oxygen demand (mgO2.L-1)
200
180
160
140
120
100
80
60
40
20
0
Blank
Control
0.10%
0.50%
2%
5%

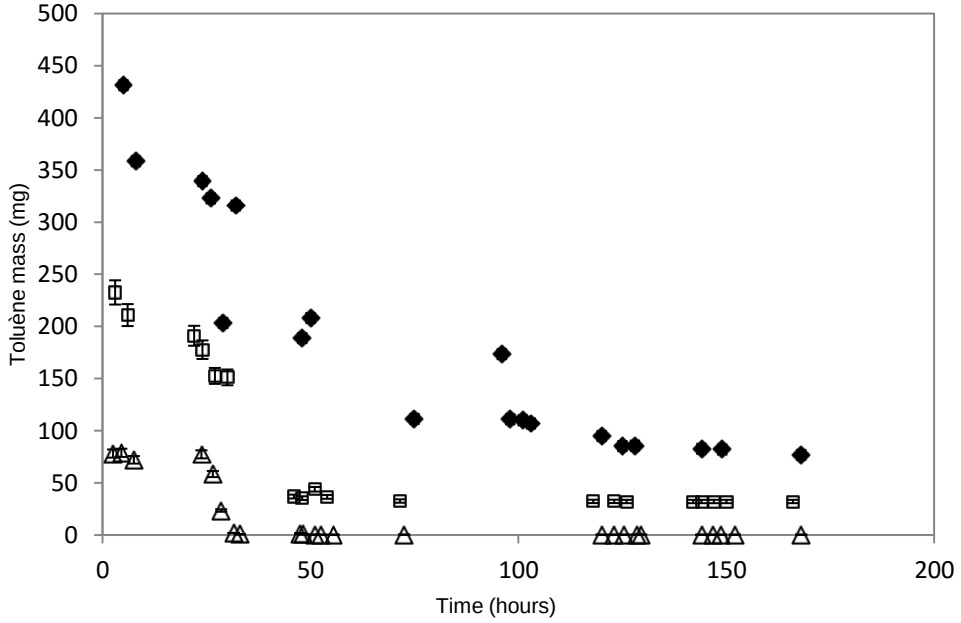

1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Toluene mass (mg)
0
2
4
6
8
Time (days)

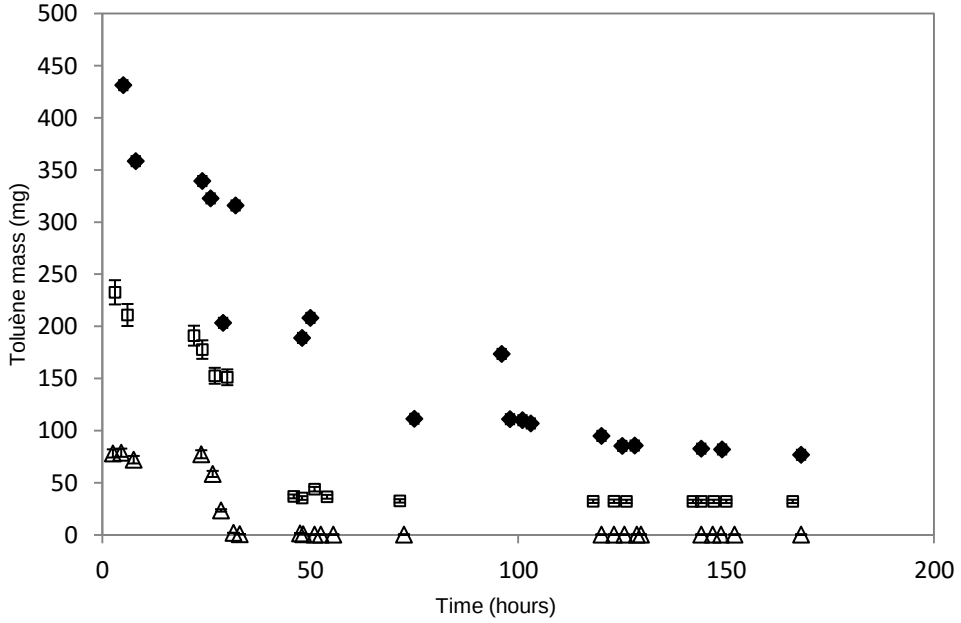

0.3
0.25
0.2
0.15
0.1
0.05
0
DEHP mass (g)
0
2
4
6
8
Time (days)


35.000
30.000
25.000
20.000
15.000
10.000
5.000
0.000
Toluene mass (mg)
0
50
100
150
200
Time (hours)


0.300
0.250
0.200
0.150
0.100
0.050
0.000
DEHP mass (g)
0
50
100
150
200
Time (hours)


0.300
0.250
0.200
0.150
0.100
0.050
0.000
DEHP mass (g)
0
50
100
150
200
Time (hours)


500
450
400
350
300
250
200
150
100
50
0
Toluène mass (mg)
0
50
100
150
200
Time (hours)


500
450
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150
100
50
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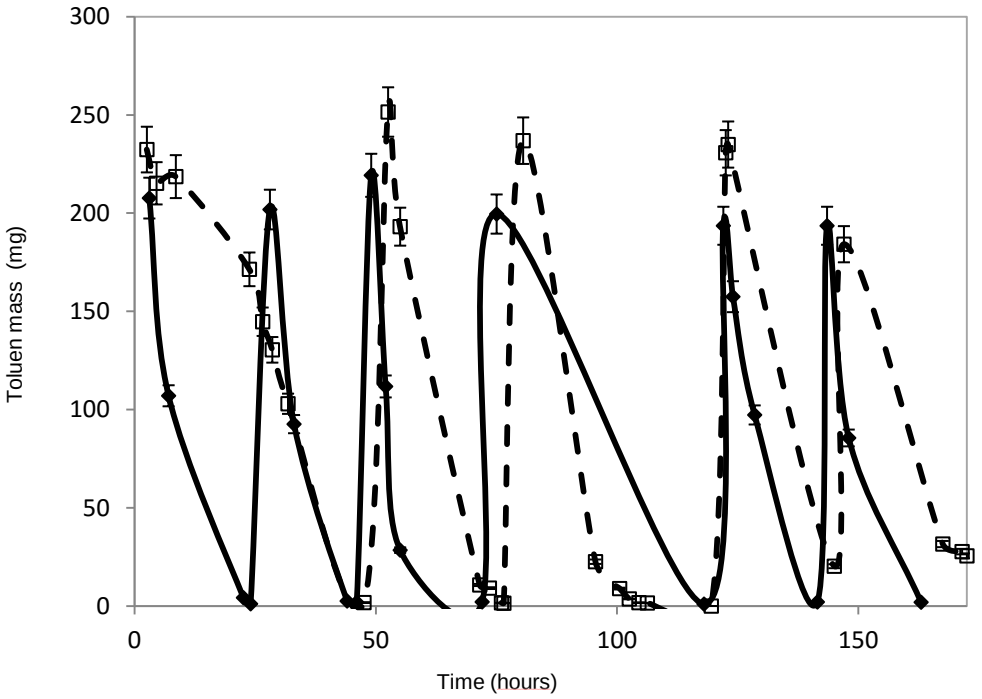
Toluène mass (mg)
0
50
100
150
200
Time (hours)


300
250
200
150
100
50
0
Toluen mass (mg)
0
50
100
150
Time (hours)