Characterization and performance evaluation of a two-phase partitioning bioreactor for volatiles organic compounds treatment in off-gas

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ABSTRACT

The treatment of the industrials off-gas strongly evolves with change of the environmental legislation on a worldwide scale. Biotechnics existing for their treatment sometimes present limits for some volatile organic compounds (VOC) such as BTEX because of their poor water solubility. The use of two phase-partitioning bioreactors (TPPB) is an interesting alternative in this case. In this work, a laboratory scale TPPB (water / silicone oil) was monitored at high level of Isopropropylbenzene (IPB) air pollution (7g/Nm^3) and a flow of 1 VVM. We focused ourselves on the inoculation with the strain *Rhodococcus erythropolis T 902.1.* We showed that the increase of the inoculums size to 5 g DM/l. induces a better initial abatement of the pollutant, however performances of the TPPB decrease quickly because of cellular mortality. The use of a smaller inoculum (0,2g DM /l) seems to be a good compromise to observe progressive improvement of the IPB abatement with adaptation of biomass. The TPPB was followed during 38 days in order to confirm its potentialities and characterise its evolution. We showed that the performances of the TPPB are maintained with an elimination rate near to 63 % for IPB polluted air (7 g/Nm^3) and punctually reach 92 %. The biomass grows gradually and stabilizes itself around 10 g/l. With fluorescent double stain Rhodamine/ propidium iode, we also shown that cellular viability strongly evolve: cellular viability was low (30 %) in the first operating hours but is quickly increased after adaptation to IPB (80%); we also suggested an endorespiration phenomenon in the bioreactor.

In this work, we could confirm the previously estimated elimination performances of the two- phase partitioning bioreactor with silicone-oil as second phase. Elimination rate of a monoaromatic compound at high concentration (7 g / Nm^3) can be maintained between 240 g /m^3.h and 360 g /m^3.h. in the TPPB.

1 INTRODUCTION

Numerous polluting organic compounds are released by human activities and persist in the environment, because of their low solubility in water and their high concentration.

Monoaromatic hydrocarbons such as benzene are produced in large amounts, and used in fuels, as solvents and as starting materials for the production of plastics, synthetic fibres and pesticides (Budavari *et al.*, 1996).

Monoaromatics have become prevalent environmental contaminants, and thirty of them are on the «EPA Priority Pollutant List» (1996). Eleven of these compounds are in the top 100 chemicals on the Priority «List of Hazardous Substances» published by the Agency for Toxic Substances and Disease Registry (ASTDR, 1997).

In recent years, biological techniques have been applied more frequently to control these emissions, because they eliminate many of the drawbacks of classical physical-chemical techniques. Disadvantages of usual air treatment techniques are high-energy costs (incinerators), the use of expensive chemicals that may require special operational safety procedures (chemical scrubbers) and the generation of waste products such as spent chemical solutions or spent activated-carbon (Van Groenestijn *et al.,* 2005; Davidson *et al.*, 2003). Biological methods involving biofilter has been shown to be promising alternatives compared to the traditional technologies for the control of many gaseous pollutants (Rene *et al*., 2005). Bio filters present however several limits such as the ripening period of the bio filters during which cells proliferate to the point where the bed can be used and their restriction to the treatment of low VOC (volatile organic compounds) concentration (below 1g / Nm^3). This is partly due to the poor water-solubility of gaseous pollutants. Some researches were carried out in order to improve transfer and solubility of hydrophobic pollutants during biological treatment. Budwill and Coleman (1997) showed the positive effect of silicone-oil addition on the biodegradation rate of n-hexane vapours in peat-based bio filters. Cesàrio *et al*. (1997) showed an enhancement of the toluene mass transfer rate by a factor of 1,1 using a dispersion containing 10% (v/v) FC40 solvent and a twofold oxygen transfer rate.

More recently, many researches were devoted to the Two-Phase Partitioning Bioreactors (TPPB) as a new technology for xenobiotic degradation, The TPPB. concept has been demonstrated to be effective for the degradation oh high levels of organic compounds (Daugulis *et al*., 2001). Dumont and Delmas (2003) reviewed the mass transfer enhancement of gas absorption in oil-in-water systems and conclude that our understanding, of the influence of oil addition on the mass transfer parameters k_L and a could be improved.

By improving the oxygen and pollutant mass transfer and reduce the inhibitory substances by lowering their concentration in the aqueous phase, the elimination capacity reported for two-phase partitioning bioreactor often exceeded the performance

of bio filters. For nonexhaustive example, the elimination capacity quoted in some studies are summarized in Table 1.

Table 1.
Some examples of elimination capacity quoted in literature for some compounds

Compounds	**Microorganism**	**Elimination capacity (EC) g/m$^3_{réact}$.h**	**Reference**
Hexane	Pseudomonas aeruginosa	135	Muñoz *et al.* (2006)
HAP (naphtalène et phénanthrène)	Sphingomonas aromaticivorans	238	Daugulis and Janikowski (2002)
Benzene	Alcaligenes xylosoxidans Y234	291	Yeom and Daugulis (2001)
Toluene	*Alcaligenes xylosoxidans*	727 727	Daugulis and Boudreau (2002)

To compare, a recent study (Arriaga *et al*., 2006) reported in a fungal biofilter supplied with silicone-oil quote EC of 100 g hexane /m^3.h, however higher than those reported in classical fungal and bacterial biofilters (60g/m$^3_{react}$.h). The open nature of biofilters limits the control of parameters, inoculation with a single chosen species may thus fail (Devinny *et al.*, 1999). In a TPPB, the best control of parameter permits the inoculation with an adaptated strain. In a similar way, the acclimatation in compost biofilters treating gasoline vapors, was much more rapid when they were inoculated with adaptated culture but do not affect ultimater removal efficiencies (Wright *et al*., 1997; Leson and Smith, 1997). In this study, we review the merit and limitation of a water / silicone oil TPPB used for treating isopropropylbenzene (IPB) gaseous vapours at high concentration. We also consider the influence of various quantities of inoculum on the initial evolution of EC.

2 MATERIALS AND METHODS

2.1 Microorganisms and cultivation

The *Rhodococcus erythropolis* strain was obtained from the collection of the Walloon Centre of Industrial Biology (C.W.B.I.; Belgium).

All the substrates and other chemicals were purchased at VWR international (Leuven, Belgium) or Aldrich (Bornem, Belgium).

The culture of *Rhodococcus erythropolis* in 868 medium (glucose 20g/l.; casein peptone 20 g/l; yeast extract 10 g/l) is harvested after 64 hours (optical density 600 nm =1,4). The inoculum for the biological reactor is obtained by centrifugation of various volume of this culture in function of experiment. The pellet obtained is washed twice and diluted in 200 ml saline water (9g/l NaCl). The inoculum is then introduced into the bioreactor where the medium for biodegradation is composed of silicone oil (10% V/V) and aqueous medium M284 (90 % V/V) whose composition is : Na_2HPO_4 17,7g/l; NaH_2PO_4 24,33 g/l (buffer pH 7); NaCl 4,68g/l ; KCl 1,49g/l; NH_4Cl 1,07g/l; Na_2SO_4 0,43g/l; $MgCl_2$. $6H_2O$ 0,20g/l ; Na_2HPO_4 $2H_2O$ 40mg/l ; $CaCl_2$. $2H_2O$ 30mg/l Fe(III)NH_4citrate 4,8mg/l; $ZnSO_4$.7 H_2O 0,144 mg/l ; $MnCl_2$.4 H_2O 0.1 mg/l ; H_3BO_3 0,062 mg/l ; $CoCl_2$.6 H_2O 0,19 mg/l; $CuCl_2$.2 H_2O 0,017 mg/l ; $NiCl_2$.6 H_2O 0,024 mg/l ; Na_2MoO_4.2 H_2O 0,036 mg/l; ethanol 1g/l.

2.2 Bioreactor and assembly

The stirred bioreactor used for biodegradation (LSL Bio Lafitte BL06.1, Saint German en Laye, France) described by Aldric (2005). Its reactional volume reaches 4,5l and the stirring speed was maintained at 600 rpm. The assembly is schematized in figure 1.

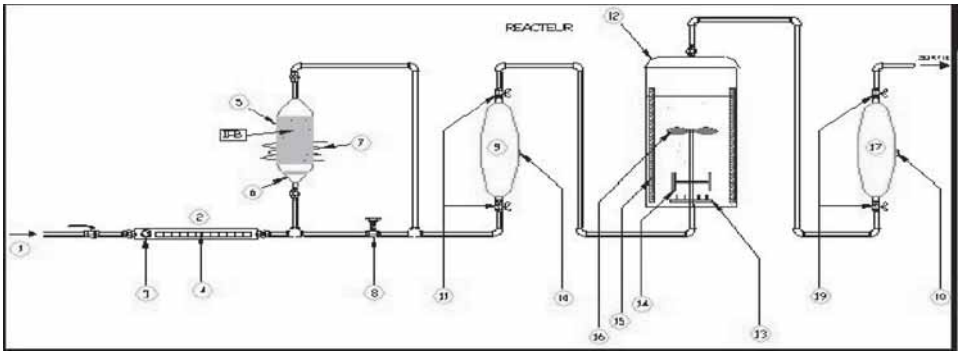

Figure 1. Schematic of the laboratory scale TPPB. A flow-meter (2) allows the control of the gas flow by means of a control valve (3). A bottle (5) containing pollutant (IPB) allows the generation of the polluted effluent with a temperature control (7). A valve is used to dilute the polluted effluent and to control the concentration at level of the sampling bubble preceding the bioreactor (9). A septum (10) is used to sample gas. The stirred bioreactor is the seat of the biodegradation (12). A sparger (13) is used to allow a diffusion of little bubbles of gas effluent within the bioreactor. Stirring and mixing are carried out by means of two agitation modules : a TD4 module (14) and a helicoidal module (15). Baffles (16) avoid the formation of vortex within the bioreactor. Lastly, a sampling bubble permits to measure the residual concentration at the exit of the bioreactor.

The IPB concentrated gas is generated by stripping within a thermostatized glas bottle. The gas flow is permanently controlled by a flow meter and fixed at 4,5 l/min.

The concentration of IPB in the gas coming in the bioreactor is controlled by an adjustable mixture between polluted gas and air and maintained at 7 g / Nm^3.

2.3 Sampling and analytical methods

Gas samples are regularly taken from each bubble of sampling as well as in the liquid reactional medium. IPB concentration was estimated thanks to a Perkin Elmer headspace sampler HS 40 XL (for liquid samples) and a gaz chromatograph Hewlett Packard 5890 equiped with a Alltech INC. Deerfield EC-WAX column and flame ionisation detector (for gas-samples). Temperatures of the injector, column and detector were respectively 153, 150 and 250 °C.

2.4 Cellular viability

Liquid samples are regularly taken from TPPB and diluted to approximately 106 UFC/ml. 1ml was then centrifuged (8000 rpm; 10 min), the pellet was twice washed with sterile saline water (9g/l NaCl). The pellet was double stained with 5µl of Rhoda mine 123 (1,25 mM) and 5µl of propidium iode (1,25mM), homogenized and incubated 5 minutes at ambient temperature. The cellular vialbility can be evaluated by a fluorescent microscopy, viable cells appear green and nonviable cells appear red. The proportion of viable cells were estimated by enumeration of red and green cells in 5 microscopic fields of vision / sample.

3 RESULTS AND DISCUSSION

3.1 Influence of inoculum density on the removal efficiency

In this experiment, the TPPB was inoculated with several inocula in order to evaluate impact of initial cellular biomass on the performances of bioreactor during the firsts four days. Indeed, the ratio pollutant concentration / adapted micro-organisms concentration could be an important factor when the TPPB is started.

According to the data shown in Figure 2, it appeared that the use of a high size of inoculum (2,4 g DM / l) allows reaching initial removal efficiency near to 95 % but a reduction in RE is subsequently observed during the first 4 days. On the other and, for a lower size (respectively between 0,2 and 1,53 g DM/ l) the RE was maintained with a low value (around 80%) during the first 4 days. Singularly, smallest size of inoculum (0,2 g DM / l), allows to reach the highest RE after 4 operating days (84 %).

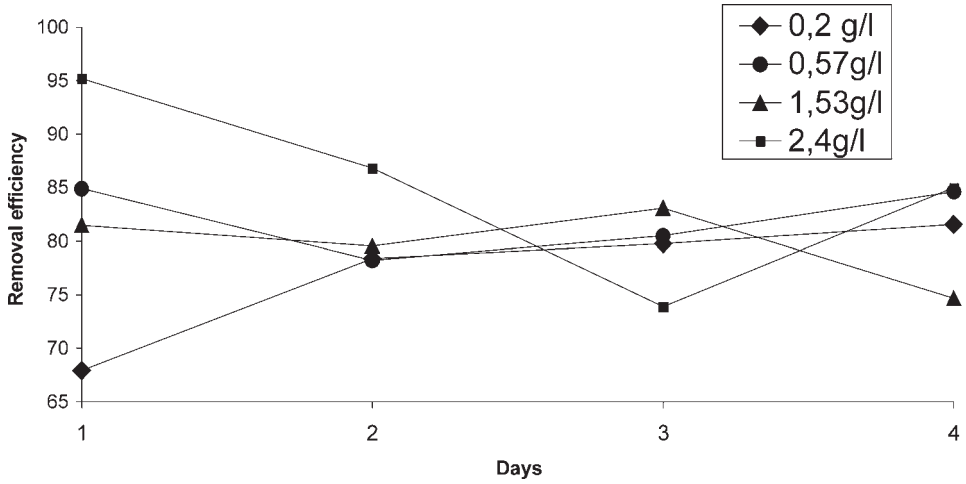

Figure 2. Influence of size of inoculums on the performance of a TPPB removing IPB.

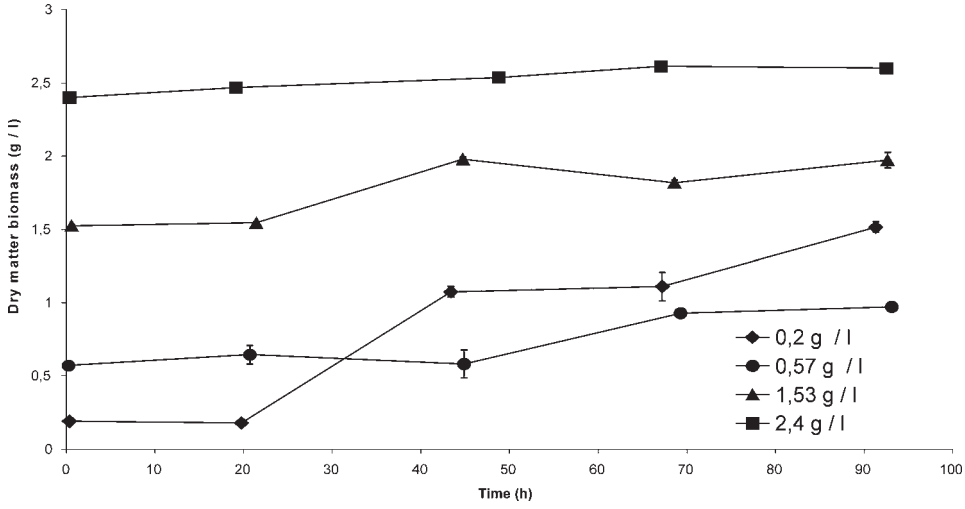

Figure 3. Evolution of biomass dry matter during the first 4 days of experimentation for various size of inoculum.

Figure 3 clearly indicates that the cellular multiplication is inversely proportional to the size of the inoculum. When the inoculum size is high (beyond 1,5 g/l), no beneficial effect can be observed, neither on the cell multiplication, nor on the removal efficiency. However, under the same conditions, a TPPB inoculated with an inoculum

size of 0,2 g/l reach 3,75 g/l and a RE maintained at 85 % after 10 operating days (results not shown). Small inoculum (0,2 g/l) seems to be the best compromise to quickly reach high RE and an adaptation of the biomass to high IPB concentration.

3.2 Evaluation and evolution of the performances of the TPPB

The performances of the TPPB were evaluated while following the elimination flow of IPB (during 38 days). The TPPB was first continuously sparged during the first four days with an inlet gaz flow (7g/Nm^3 IPB; 1 VVM) (phase 1). Then, the TPPB was sparged only during the day (phase 2) because of the too much low RE during the first phase. Lastly, the TPPB was again continuously sparged between day 30 and day 38 to evaluate the performances under extreme conditions (phase 3).

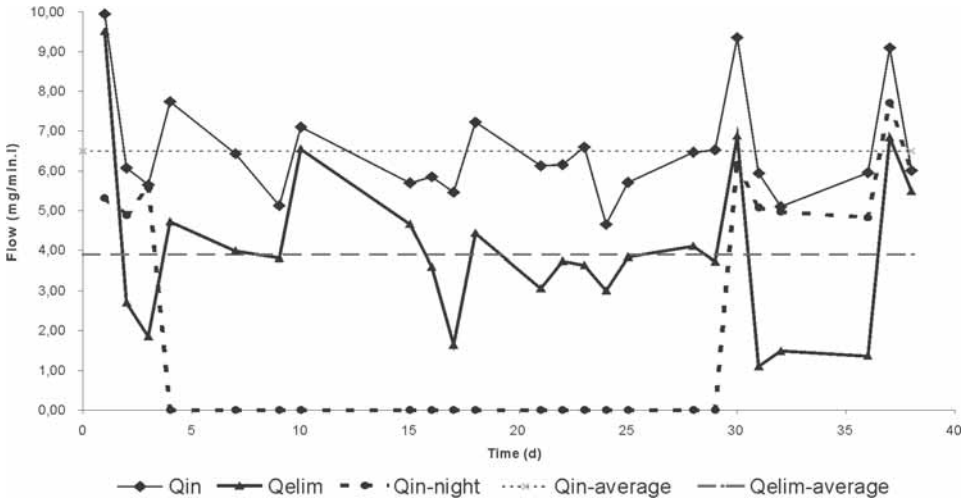

Figure 4. Evolution of the flows within the bioreactor Qin (rhombus) : IPB flow coming in TPPB; Q_{elim} (triangle): IPB elimination flow (mg/min.l). Small dotted lines and large dotted lines represent respectively the average of Qin and Q_{elim} during phase 2.

Figure 4 shows the performances evolution for a TPPB during each phase. It should be specified that le TPPB is used in the limiting predetermined conditions (Aldric *et al*., 2005), however, under these conditions, the removal efficiency is supported at approximately 63 % and punctualy reach 81% and 92 % with twelfth and thirteenth days, the elimination flow during phase 2 is thus estimated at 4mg/l.min (240g/m$^3_{react}$.h). On the other hand, when starting the third phase, the removal efficiency fall to 25 % (elimination flow below 2mg/l.min) at day 31 but follows a readadaptation at the end of experiment.

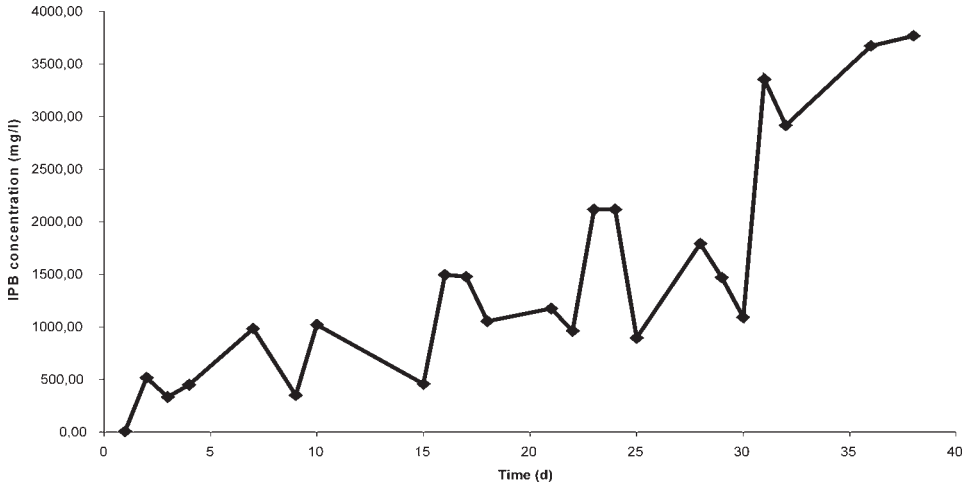

Figure 5. Evolution of IPB concentration in water-silicone oil media.

IPB concentration in the two-phase media (Figure 5) increases gradually during phase 2 and stabilizes itself between 1000 and 1500 mg/l. The absorbtion of pollutant is indeed followed of a consequent biodegrdation since the accumulation of IPB in the TPPB is limited. Nevertheless, continuous sparging with IPB gas flow (night and day; phase 3) increases strongly the IPB accumulation. This leads to a reduction of driving force term ($C_L^0 - C_L$) resulting from well-known equation 1, this can explain the reduction of removal efficiency observed.

$$\frac{dC_L}{dt} = K_L a.(C_L^{\ 0} - C_L) \qquad \text{eq. 1}$$

3.3. Evolution of biomass

Growth of *Rhodococcus erythropolis T 902.1* within the TPPB and viability of cells constitute significant parameters to evaluate the influence of the IPB load on the biomass.

Figure 6 shows a high growth of the biomass during the first 19 days, from 0,3 g/l to 12,75 g/l. Thereafter, the biomass is stabilized between 9 and 11 g/l as from day 20 until day 38. Figure 6 show a very low viability of the cells when the TPPB is starded, only 15h after inoculation with washed biomass. Cellular viability increases then continuously to reach 84 % after 7 operating days and to be maintained between 68 % and 78 % during phase 2. When phase 3 is started (day 30), the biomass seems to undergo a shock probably induced by the increase of IPB concentration in two-

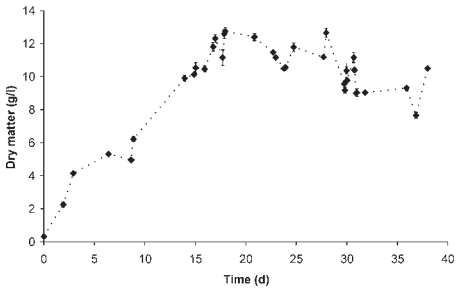

Figure 6. Evolution of biomass dry matter (DM)

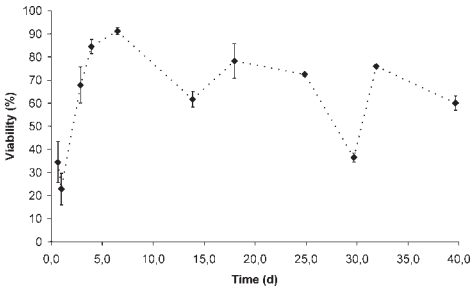

Figure 6. Evolution of cellular viablity

phase media, however the biomass seems to subsequently readapt with the news operating conditions.

The stabilization of the biomass as well from the quantitative point of view (dry matter) as qualitative (viability) suggest a cellular metabolism more directed towards the biodegradation and the endorespiration phenomenon that towards the cell multiplication.

4 CONCLUSIONS

The following conclusions can be drawn from the results presented in this study

(1) The use of a small inoculums size (0,2 g DM/l) is preferable with the use of more significant sizes because of a better adaptation of the biomass to IPB load.
(2) The use of a TPPB water-silicone oil to treat hight concentration IPB flows is confirmed. For an IPB load near to 390 $g.m^{-3}.h^{-1}$, an IPB average elimination flow of 240 $g.m^{-3}.h^{-1}$ can be obtained and maintained during 31 days of noncontinuous operating conditions, in addition the biomass remains functional.
(3) The limits of the TPPB seem to be reached when the TPPB is continuously fed with strongs IPB flows and concentration, although the biomass seems to relatively readapt after the shock.

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