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Sludge acclimation and preliminary tests prior to support material inoculation in a biofilter

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ABSTRACT. A crucial variable for the successful operation in biofilters is the selection, acclimation and activation of the microorganisms that will degrade the contaminants. The purpose of this study is to apply a procedure for the acclimation and activation of biomass of different origin (with or without an additional carbon source). In order to obtain adapted microorganisms, sludge was collected from one wastewater treatment facility and from a synthetic resin-producing industry. Two different sediments samples were also collected from two rivers that pass through industrial areas. After sedimentation of the solid phase of each sludge, the liquid phase was transferred into glass bottles for biomass activation. This activation has been carried out with and without glucose in different doses and the biomass has been acclimated to an organic contaminant (toluene) by adding small amounts of the compound with a syringe. The contaminant consumption rate has been determined by measuring its amount at the beginning of each addition and subsequent to 24 hours. The adapted biomass will be used to inoculate a future biofilter for treating toluene.

1 INTRODUCTION

A great variety of organic compounds such as toluene are widely used in industry as they provide the starting materials for the production of many everyday products. Their emissions to environment can be avoided by applying eco-friendly and effective technologies such as biofiltration.

The biomass responsible for the degradation of the gaseous contaminant fed to the biofilter can be supplied by the support material itself (Barona *et al*., 2004). Nevertheless, the filter-bed inoculation with active microorganisms is a frequent and necessary prerequisite for successful operation (Kennes and Thalasso, 1998; Kennes and Veiga, 2001). Consequently, prior to the inoculation itself, the proper selection, acclimation and activation of the microorganisms is crucial to ensure the successful operation of the biofilter

Although pure strains of microorganisms can be purchased, mixed cultures can be obtained from the sludge originated in wastewater treatment plants or similar origins

(Prado *et al*., 2003; Prado *et al*., 2005). Once the biomass is in the laboratory, the best process to grow specific strains is batch or microcosms culture (León *et al*., 1999). Microcosms experiments are also useful for estimating the qualitative evolution of the biological activity of the biofilter, especially when the bioreactor performance is near but lower than 100% (Acuña *et al*., 1999).
The purpose of this study is to apply a procedure for the acclimation and activation of biomass of different origin in order to inoculate these cultures to a future biofilter for treating toluene.

2 MATERIALS AND METHODS

2.1 Preliminary experiments

All the experiments in this study were carried out in glass bottles which were provided with a mininert teflon valve and a synthetic rubber septum (Figure 1). In order to determine how many times a valve septum can be perforated before replacement, two compounds were selected for these previous leaking experiments: toluene, as the target contaminant in this study (vapour pressure 2.9 kPa at 20 ^{o}C) and, acetone, as a highly volatile compound useful for leakage tests (vapour pressure 24 kPa at 20 ^{o}C). The preliminary experiments were performed by adding 1 µl of toluene or acetone to the bottles with a syringe (Hamilton, USA). All the bottles were previously filled with 14 glass balls for gas homogeneity. Each bottle septum was perforated 8, 10, 12, 14 and 20 times with the syringe and possible leakage was determined by weighting every 12 hours. During these experiments the mininert valve was in the open position and the bottles were placed on an orbital shaker (Lab-line Instruments) at 175 rpm.

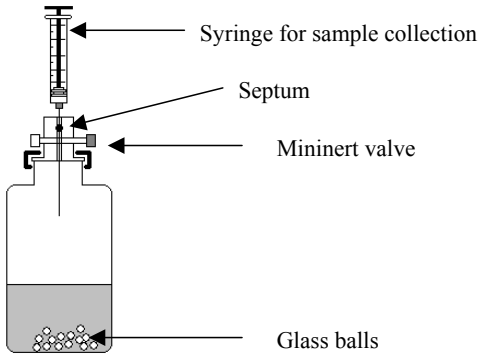

Figure 1. Sampling technique from the bottles provided with Mininert valves.

2.2 Microcosm experiments

Initial bacterial consortia were collected from the sludge of one wastewater treatment facility (W), from the sediments of two rivers that pass through industrial areas (R1 and R2) and from a synthetic resin-producing industry (F).
After sedimentation of the solid phase of each sample, the liquid phase was transferred into the glass bottles provided with Mininert valves for biomass activation. The sediments collected from the rivers were previously washed with a 0.1% NaCl solution. A nutrient medium (Table 1) was used for enriching and maintaining the four cultures

and 25 ml of nutrient solution were mixed with 25 ml of the transferred liquid phase in the glass bottles.

The activation experiments were carried out by previously adding glucose to the four samples before toluene addition. A dose of 0.03 g of glucose was added to each sample and the consumption of glucose was measured after 24 hours by using Keto-Diabur-test 5000 reactive strips. A new addition was only repeated when the substrate was consumed totally. When two consecutive additions were consumed in 48 hours, the activation experiment was concluded.

Table 1. Composition of the nutrient medium.

Macronutrient	Concentration (g/l)
KH_2PO4	0.20
K_2HPO_2	0.80
$MgSO_4$ $7H_2O$	0.05
$CaSO_4 2H_2O$	0.02
$FeSO_4 7H_2O$	0.02
$(NH_4)_2SO_4$	1.00

Micronutrient	Concentration (mg/500ml)	Micronutrient	Concentration (mg/500ml)
$FeCl_2$ 4 H_2O	1000	$(NH_4)_6Mo_7O_{24}$4 H_2O	45
$CoCl_2$ 6 H_2O	1000	EDTA	500
$MnCl_2$ 4 H_2O	250	Na_2SeO_3	50
$CuCl_2$	30	Ni Cl_2 6 H_2O	25
$ZnCl_2$	25	HCl 36%	1
H_3BO_3	25	Resazurine	250
HCO_3Na	1000		

After this activation period, two sets of acclimation experiments were carried out; one of them used the previous samples activated with glucose and the other set used samples as collected. These tests were carried out at 28 ^oC by injecting 1 µl of toluene per day into the glass bottles capped with Mininert valves. The bottles containing the cultures were continuously shaken in an orbital shaker (at 150 rpm) and were opened to the atmosphere everyday for 20 minutes in order to ensure oxygen supply and to avoid carbon dioxide accumulation. After oxygenation, the bottles were capped and a new toluene dose of 1 µl was injected. In these tests, the evolution of toluene was monitored after the addition of the contaminant.

In order to quantify the decrease in toluene concentration in these acclimation experiments, a HP 6890 gas chromatograph was used. This device was equipped with two capillary columns connected in series (60 m, Hewlett-Packard HP- PLOTQ column and Molsieve column), a flame-ionisation detector (FID) and a conductivity detector (TCD). Operating conditions were: injector temperature, 150 ^oC; oven temperature, an increasing rate of 30 ^oC min^{-1} to 250 ^oC; detector temperature, 250 ^oC; Helium carrier gas, 6 ml min^{-1}. A 250 µL gas-tight syringe (SGE, Melbourne, Australia) was used for sample injection.

In order to measure the evolution of mass density, 5 ml of the sample F and 500 ml of the nutrient solution were mixed in a bottled provided with a Mininert valve and, subsequently 10 µl of toluene were injected. The absorbance of the solution was measured in a a Perkin-Elmer spectrosphotometer (Lamba 2) for 8 days. After taking a

sample for measuring purposes a new dose of 10 µl of toluene was added until next sampling.

3 RESULTS AND DISCUSSION

Leakage tests rendered different results for the two compounds studied. Valve septum in the bottles containing toluene can be perforated more than 20 times before leakages are detected; however, the acetone containing bottles should be perforated 12 times as a maximum in order for the mass losses of the compound throughout the septum to be lower than 2% during 60 hours with open valve position. This different behaviour is associated with the fact that toluene molecular weight is 1.58 times higher than that of acetone.

Differences in the acclimation period between the sludge samples (W and F) and river samples (R1 and R2) were observed. The glucose consumption was very rapid in the bottles containing samples W and F (from the industry). The samples R1 and R2 also consumed glucose but at a lower rate than the others, which is an indicative of a lower amount of active biomass.

As far as acclimation without glucose is concerned, samples W and F rendered an elimination efficiency of 100% 24 hours after the first toluene (1 µl) dose was added (Figure 1).

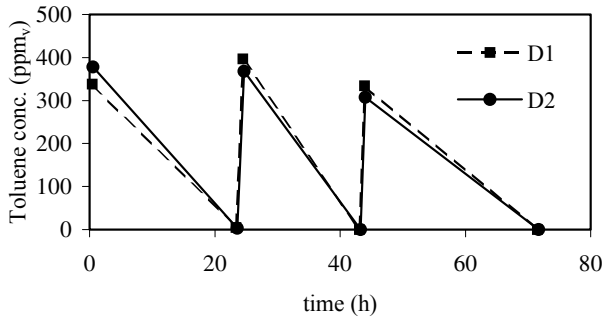

Figure 1. Toluene consumption in the bottle containing biomass from a wastewater treatment plant (W) without previous addition of glucose (D1 and D2 duplicate 1 and 2 respectively).

The biomass of samples R1 y R2 required a brief activation period when glucose was added, but a longer acclimation period was necessary when the contaminant was fed into the bottles.

As a consequence, activation with glucose is only recommended when indiscriminated biomass is to be activated, but it its addition did not render better acclimation results in this study.

The evolution of biomass density was measured only in sample F, and results are shown in Figure 2. The new additions of toluene after each sampling rendered an increase in active microorganism density. Thus, the sample F provided native microbial population

that could be used to inoculate conventional biofilters packed with synthetic or iner carriers. However, after a weeks of operation, although inoculated strains might still be present in the biofilter, other new strain will most often grow on the carrier material.

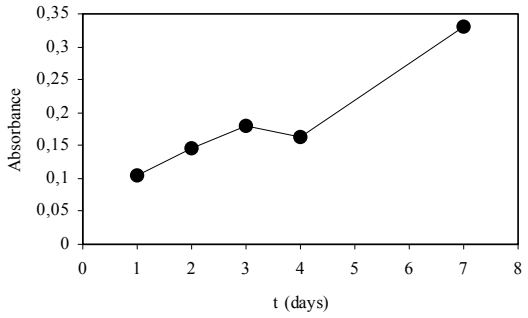

Figure 2. Evolution of biomass density for sample F.

Other analysis are currently being performed in order to complete the results shown in this study.

4 ACKNOWLEGEMENTS

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