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Start-up of biofilters: continuous acclimation of biomass for inoculation purposes

A. Barona, A. Elías, G. Gallastegi, A. Garcia
G. Ibarra-Berastegi, L. Gurtubay
* University of the Basque Country, Engineering School, Alda Urquijo s/n 48013
Bilbao, Spain, astrid.barona@ehu.es

The success of the start-up of biofilters basically relies on a proper selection and acclimation of the biomass responsible for the degradation of the contaminant. This study has undertaken a comparison between discontinuous and continuous acclimation modes to toluene. The results obtained show that the continuous adaptation mode allowed a quicker growth of biomass as the oxygen supply was ensured. It also allowed for a clearer distinction between the activated samples. In addition to determining solid content, the C and H elemental analysis rendered additional information on the evolution of biomass growth in the discontinuous mode. The sample collected in a wastewater treatment plant (W) rendered the best results in all cases. The SV/ST ratio for this sample after 1050 hours was 0.85 for continuous operation, in contrast to a value of 0.57 for the discontinuous mode. Two biofilters inoculated with the selected W sample showed a highly efficient start-up operation, as the removal efficiency of toluene immediately reached values higher than 80%. Moreover, values close to 100% were maintained over 2550 hours for an inlet toluene concentration ranging from 1.58 to 34.11 g $m^{-3}h^{-1}$.

Keywords: biotreatment, biofiltration, biomass growth, continuous and discontinuous acclimation

1. Introduction

Biological technologies have proven to be a reliable “green” alternative to other treatments because biodegradation occurring in bioreactors results in the generation of simple molecules with no, or limited, effect on human or environmental health.
The biomass responsible for the degradation of the gaseous contaminant fed into a biofilter can be supplied by the support material itself (Barona et al, 2004). Nevertheless, filter-bed inoculation with active microorganisms is a frequent and necessary prerequisite for successful operation (Kennes and Thalasso, 1998; Kennes and Veiga, 2001). Prior to the inoculation itself, the proper selection, storage, acclimation and activation of the microorganisms is crucial to ensure long-lasting bioreactor operation. In fact, Prado et al. (2005) proved that the prior biomass concentration and biomass adaptation of the inoculum dramatically affected the start-up

and performance of conventional biofilters treating methanol during the first stages of operation.

Likewise, simple analytical techniques are needed for biomass growth detection. Appart from the methods developed by microbiologists to estimate the activity of the biomass, other simpler analyses, such as carbon balance analysis and volatile suspended solid content are also very helpful for quantifying biomass concentration and adaptation (Prado et al., 2005; Xi et al., 2006). Bearing in mind that biofilter controlling operators require simple and quick techniques for everyday operation, biomass carbon content or total biomass can be measured as the percentage of volatile solids (Moe and Irvine, 2001; Kim and Sorial, 2007). These simple techniques are very useful but they have obvious disadvantages, such as no discrimination between living or dead biomass, or no accounting for changes in microorganism physiology.

Thus, the objective of the study presented here is to establish a simple procedure for previously selecting samples to be used as inoculum for toluene biofiltration purposes. Continuous and discontinuous (batch) modes were investigated for activating three samples collected in contaminated locations. Two simple and quick analytical techniques (solid content and elemental analysis) were used to achieve biomass growth. Considering that the ultimate purpose of activation is to obtain a suitable inoculum, two biofilters will be inoculated with one previously selected sample from amongst the three collected.

2. Materials And Methods

2.1 Source of the microorganisms and media composition

Three sludge samples containing the original biomass were collected in a wastewater treatment plant (W), in a small river close to a petrochemical company (P) and near a synthetic resin-producing industry (F). The P and F samples were first cleaned with a 0.1% NaCl solution. The W sample collected from the aerobic tank of a local plant treating urban and industrial wastewater did not require further cleaning.

After sedimentation of the solid phase in each sample for 2 hours, the liquid phase, where a higher amount of microorganisms is expected to be found, was transferred into glass bottles for further experimentation. A nutrient medium (Cano et al., 2005) was used for enriching and maintaining the cultures. Thus, 25 ml of nutrient solution were mixed with 25 ml of the transferred liquid phase in the glass bottles.

2.2 Batch assays (discontinuous operation)

Batch assays tests were carried out at 25 ^{o}C by injecting toluene into 156 ml glass bottles capped with Mininert valves and containing 1:1 (v:v) of the nutrient solution and the liquid phase of each sample. Four replicates of each sample and one blank were prepared. Toluene was added for 1050 hours (42 days) at a daily rate of 2 μL for the first 400 hours, 4 μL for the following 400 hours and 8 μL for the final 250 hours. The bottles were continuously shaken in an orbital shaker (at 150 rpm) during experimentation and were exposed 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 was injected.

In these batch assays, the evolution of the ratio between the volatile solid content (VS) and the solid total content (ST) was measured over time. The solid total content was determined by drying the samples at 105 ^{o}C and the volatile solid content, by drying the samples at 550 ^{o}C. The elemental content (C and H content) of the dried samples at 105 ^{o}C was also determined over time in a CE 440 Elemental Analyzer. The sampling for these analyses was carried out alternately in the four replicates so as not to cause major disruption to the systems.

2.3 Continuous operation

Biomass activation was also carried out in continuous operation for 1050 hours at 25 ^{o}C. The tests were performed in three-litre vessels or reactors with one entrance tube and one exit tube on the upper part. A 1:1 (v:v) original sample:nutrient solution was used. The entrance tube was submerged in the liquid and it was used to feed a contaminated air flow (2.5 l min^{-1}) with a toluene concentration ranging from 15 to 50 ppmv (from 2.46 to 8.23 g m^{-3}h^{-1}). The air flow was previously bubbled into water for saturation before mixing with toluene. The systems were additionally shaken with a Teflon shaker at 200 rpm. The evolution of VS/ST and the toluene inlet and outlet concentration over time were checked.

2.4 Biofilter operation

Based on the previously obtained results, the most promising sample was selected for use as inoculum in two biofilters. The packing material selected was made up of composted pig manure and sawdust and has already been used in previous work (Elías et al., 2002). An amount of 400 g of the packing material was mixed with 100 ml of the selected inoculum in a tray. This operation was repeated with a total amount of 1200 g of the support material in order to fill the two biofilters. Each biofilter consisted of two 1.5 l modules. The outline of the pilot plant is shown in Figure 1.

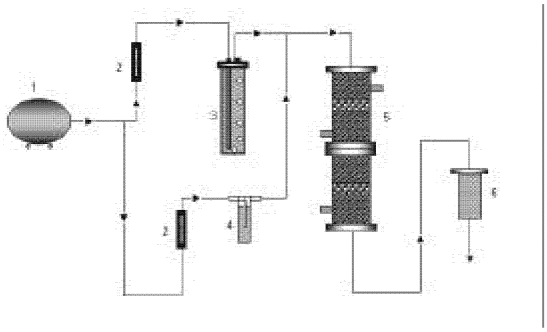

Figure 1. Outline of the biofiltration system (the same system operated for biofilter 1 and 2). 1. Air-compressor 2. Flow meter 3. Humidification chamber 4. Toluene vessel 5. Biofilter with two modules 6. Active carbon chamber.

The contaminated gas flow for one of the biofilters (biofilter 1) was 1.3 l min^{-1} (residence time of 138 s), and for the other biofilter (biofilter 2) it was 1.1 l min^{-1} (residence time of 163 s). The inlet toluene load ranged from 29 to 400 ppmv for

biofilter 1 and from 22 to 370 ppmv for biofilter 2. These slight differences between the two inlet loads were due to technical difficulties in accurately controlling the inlet concentration.

2.5 Analytical methods

In the discontinuous acclimation systems, air samples were withdrawn from the head space of the systems by means of a gas-tight syringe. The samples were analyzed on a HP 6890 gas chromatograph (GC) equipped with two capillary columns connected in series (60 m, Hewlett-Packard HP- PLOTQ column and Molsieve column), and on a flame-ionisation detector (FID). Operating conditions were: injector temperature, 150 ^{o}C; oven temperature, an increasing rate of 30 ^{o}C min^{-1} to 250 ^{o}C; detector temperature, 250 ^{o}C; Helium carrier gas, 6 ml min^{-1}. A 250 µL gas-tight syringe (SGE, Melbourne, Australia) was used for sample injection.

In the continuous acclimation mode and biofilter operation, the toluene content in the upper and lower sampling ports of the bioreactor was determined in a microgas chromatograph (microGC CP 4900) equipped with auto-sampling injection mode and a TCD detector.

3. Experimental Results And Discussion

3.1 Batch assays (discontinuous operation)

Batch assays showed that the three samples were able to degrade more than 96% of the toluene fed when 2 and 4 µL were added (data not shown on behalf of brevity). However, when the contaminant addition was 8 µL, the W sample maintained the highest degradation amount on a daily basis. On the other hand, the lowest value was recorded for F sample (only 60% of the toluene fed on a daily basis was degraded). In general terms, and according to these preliminary results, samples W and P showed a very similar response in these assays, with further information being required for selecting the best inoculum.

Although the amount of biomass is usually related to the degradation rate of a target contaminant, it has been proven that biodegradation efficiencies are not directly proportional to the biomass present in all cases (Villaverde et al., 1997). Bearing in mind that a gradual and controlled increase of biomass is deemed necessary for achieving the long-term performance of biofilters, the ratio between volatile solid content (VS) and total solid content (TS) over time for the three samples is shown in Figure 2. The SV/ST ratio slightly increased for samples P and F, whose evolution over time run parallel. The sample W achieved a clear increase of that ratio and clearly differed from the other two samples.

Thus, another alternative to correlate degradation efficiency and biomass semi-quantification was to measure the carbon and hydrogen (C and H) elemental content. Assuming that the inorganic carbon content remained constant throughout experimentation and that the sole carbon source for the biofilter was toluene, the carbon balance analysis was proven to be effective for biomass estimation (Xi et al, 2006). Theoretically, three carbon portions are distinguished. One portion of the carbon entering the system (as toluene) is emitted in the form of CO_2 (not measured in this

study). Another portion is discharged by the liquid leachate, which is not the case in these batch assays in semi-liquid phase. The third portion is the accumulated carbon portion, which is related to the biomass amount accumulated in the system.

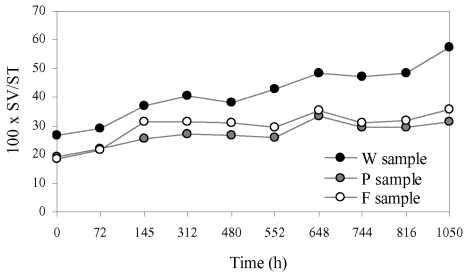

Figure 2. Ratio between volatile solids and total solids for the three samples over experimentation time in the discontinuous acclimation mode

In this study, the total carbon content measured in the previously dried suspension can be directly related to the third portion or to biomass accumulation. Thus, the evolution of total carbon content is illustrated in Figure 3. The original total C content of sample W was 2.89% and this value was increased to 11.66% after 1050 hours of operation (that is, the final C value was 4.03 times higher than the initial one). However, the relative increase of total C content was surprisingly higher in samples P and F, as the increase was 11.49 and 5.63 times higher, respectively, in comparison to the initial amount (0.87 and 1.35%, respectively). In spite of these comparative data, a clear difference was achieved between sample W and samples P and F in the last 300 hours of experimentation. From 750 hours onwards, the C content of sample W showed a slight tendency to continue increasing. On the other hand, samples P and F reached a constant C content value, which revealed that biomass growth was halted in these two cases.

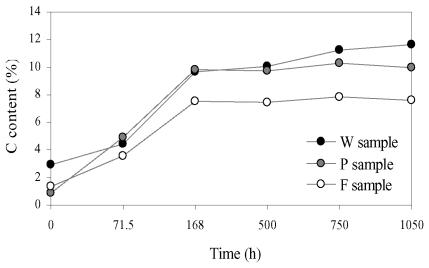

Figure 3. Evolution of total C content in the discontinuous acclimation mode.

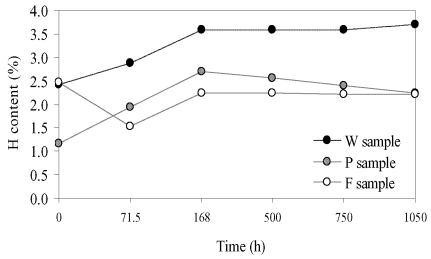

Figure 4. Evolution of total H content in the dicontinuous acclimation mode.

The evolution of H content over time in the dried samples is presented in Figure 4. Assuming that a typical cellular composition for a heterogeneous microorganism can be represented by $C_5H_7O_2N$ (Kim and Sorial, 2007), the H content increase was attributed

to an increase in the amount of biomass (dry measure). Thus, the H content in sample W slightly increased over experimentation time. By contrast, the H content in sample F was constant after 168 hours of operation, decreasing in sample P over the same period of operation.
Thus, based on these batch experimental results, the ratio between VS and TS rendered a clear difference between sample W and the other two samples. In addition, total C content showed that the biomass growth for samples P and F basically stopped as of 168 hours of operation.

3.2. Continuous operation

The biomass was also acclimated in continuous mode operation, which involved the continuous feeding of toluene. The SV/ST evolution over time for the three samples clearly increases until 670 hours of operation, when the toluene inlet concentration ranged from 15 to 50 ppmv (from 2.46 to 8.23 g m^{-3} h^{-1}) (Figure 5). From that moment onwards, samples F and P maintained a constant SV/ST ratio, which is indicative of an inhibition or halting of biomass growth. However, the biomass of sample W was able to continue growing after 1050 hours of operation.
Comparing the results shown in Figures 2 and 5, it can be concluded that continuous operation led to higher SV/ST values that may be attributed to faster biomass growth.

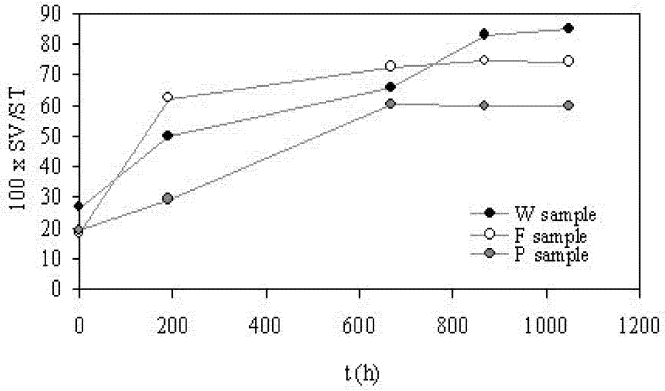

Figure 5. Ratio between volatile solids and total solids for the three samples over experimentation time in the continuous acclimation mode.

The removal efficiency measured over time (data not shown on behalf of brevity) showed that the three samples appeared to behave equally in degrading the toluene, but the data measured after 1100 hours of continuous operation revealed that W sample was the only one able to maintain increasing removal efficiencies.
Based on these results as a whole, the sample from the wastewater facility (W sample) was selected as the best inoculum for the two biofilters presented below. However, it should be mentioned that the other two samples were also able to acclimate to the toluene, although they rendered lower values for the control parameters.

3.3. Biofilter operation

The inoculation of a previously adapted biomass will accelerate and improve the performance of the start-up phase in biofilters (Prado et al., 2005). Accordingly, the previously selected sample W was continuously acclimated for 1050 hours, as explained before, and it was used as inoculum for the two biofilters. Downflow operation was selected, as it allows for better drainage (better moisture and biomass distribution) (Prado et al., 2005). The two conventional biofilters were started up and operated for 2550 hours (Figure 6 and 7). The inlet toluene load ranged from 29 to 400 ppmv (from 2.47 to 34.11 g m^{-3} h^{-1}) for biofilter 1 and from 22 to 370 ppmv (from 1.58 to 26.69 g m^{-3} h^{-1}) for biofilter 2.

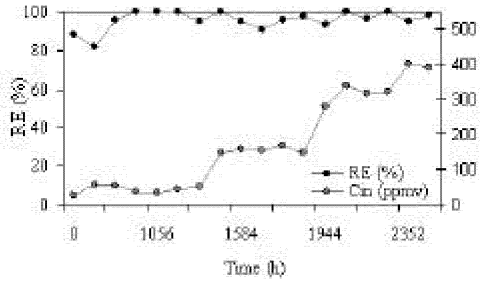

Figure 6. Response of biofilter 1 during start-up operation.

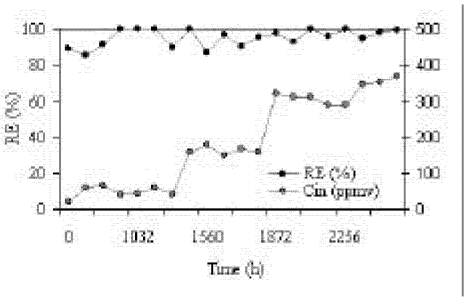

Figure 7. Response of biofilter 2 during start-up operation.

The amount of toluene retained in the support material by adsorption was previously quantified (Barona et al., 2007). Bearing in mind that both biofilters were operated for more than 2500 hours, it can be concluded that no significant adsorption took place on the support material, proving that the removal efficiency of the bioreactors was attributed to microbe activity.

Figures 6 and 7 showed that the start-up periods for both biofilters were extremely short, as the removal efficiency reached values always higher than 80% from the very first moment of operation. No significant differences were found in either biofilter, as they can be considered two replicates with the only slight difference being related to the empty bed residence time (EBRT 138 s for biofilter 1 and 163 s for biofilter 2). In both cases, stable removal efficiencies (RE) between 80 and 100% were reached, although inlet pollutant concentration was increased to 400 ppmv for biofilter 1 and 370 ppmv for biofilter 2. The high EBRT selected for the start-up of the bioreactors also had a positive influence on the high removal efficiencies.

Consequently, the previous sample selection and continuous acclimation rendered a stable biofilter with high removal efficiencies over 2550 hours of operation for moderate inlet toluene concentrations.

4. Conclusions

The use of a previously adapted inoculum may be an interesting strategy for shortening the start-up time in conventional biofilters. In this study, the acclimation of biomass in

discontinuous and continuous modes are compared based on the evolution of the ratio between the volatile solid content and total solid content over acclimation time. The results showed that the continuous adaptation mode led to quicker biomass growth and a proper selection among activated samples after 1050 hours of operation. In addition to determining the solid content, total C content showed that biomass growth for samples P and F basically stopped after 168 hours of operation in the discontinuous mode. The sample collected in a wastewater treatment plant (sample W) rendered the best results in all cases. The SV/ST ratio for this sample after 1050 hours was 0.85 for continuous operation, in contrast to a value of 0.57 for discontinuous mode.

Based on these results, the sample collected in the wastewater treatment plant was again acclimated according to the continuous procedure, being used as the inoculum for two biofilters treating toluene. The inlet toluene load ranged from 29 to 400 ppmv (from 2.47 to 34.11 g $m^{-3}h^{-1}$) for biofilter 1 (EBRT of 138 s) and from 22 to 370 ppmv (from 1.58 to 26.69 g $m^{-3}h^{-1}$) for biofilter 2 (EBRT of 163 s). Both biofilters immediately reached removal efficiencies higher than 80%, and biofilter performance close to 100% was maintained for 2550 hours.

Acknowledgements

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