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# The effect of temperature, substrate concentration and water content on carbon recovery and end-points in biofiltration

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## ABSTRACT

This biofiltration study investigated the fate of carbon as influenced by temperature, water tension and pollutant concentrations. Soil biofilters degrading toluene were operated with no supplemental nutrient addition. Rigorous control of inlet concentrations, temperature and water tensions were maintained in a differential biofilter. Temperature experiments were conducted at 20 $^{o}$C, 30 $^{o}$C and 40 $^{o}$C and water tension was varied between 10 $cm_{H2O}$, 20 $cm_{H2O}$ and 100 $cm_{H2O}$. In addition, biofilms of *Pseudomonas putida* was also investigated at similar conditions. Carbon endpoints were tracked as $CO_2$, total carbon in the liquid/solid phase and microscopic studies looked at polysaccharide production. Overall carbon balances averaged 111 % ± 9 % with the total carbon of the solid phase contributing the most to the uncertainty. Temperature and residual toluene had the strongest impact on the percentage of degraded toluene that was completely mineralized to $CO_2$ with higher temperatures and lower residual toluene giving the highest percentage mineralisation ranging from ~25 % to 92 %. Differential staining confirmed that the majority of the non-mineralised toluene was deposited in the soil as polysaccharides. This polysaccharide production would potentially contribute to biofiltration pressure drop increases over time.

**Key Words:** Differential biofilter, Non-growth, Carbon balance, Toluene

## 1. INTRODUCTION

Biofiltration provides a clean, cost effective, and environment friendly technology using mass transfer and microbial oxidation to degrade organic pollutants [1]. The biofiltration process is used effectively for treating large streams of air contaminated with low concentrations of pollutants [2]. Biofilters are packed bed bioreactors degrading pollutants through a complex and diverse mixed culture of microorganisms forming a pollutant-degrading biofilm on the porous bed medium. The ultimate fate of the degraded pollutants in biofiltration is not well understood. Attempts to close the carbon balance have been uneven, with 10-50 % of the degraded carbon often reported missing [3-6]. Given the importance of these biofilms in engineered systems, a better understanding of the metabolism pertaining to the carbon end points in various phases is paramount. This study investigated the various carbon end-points for toluene degradation in the gas, liquid and solid phases and the influence and interactions of temperature, water tension and substrate concentrations on various carbon end-points.

## 2. EXPERIMENTAL SET-UP

Laboratory scale differential biofilters (Figure 1) were operated as non-growth systems with no nutrient addition to the soil at operating temperatures of 20 °C, 30 °C and 40 °C for about 3 months per run. Other parameters such as pollutant concentrations and water tension were also simultaneously varied.

Desired gas phase toluene concentrations were generated by a diffusion tube apparatus containing liquid toluene which added toluene at a constant mass rate to a flowing air stream depending on the temperature and dimensions of the diffusion tube [7]. Approximately 8 g of soil (Parkhouse Garden Supplies) was used as a reactor bed material on the membrane arranged in a cylindrical form with a diameter of (0.053 m) and thickness of (0.003m) to minimize mass transfer limitations.

PBS solution was used in the liquid chamber which was hydraulically connected to the soil through the membrane. The liquid reservoir was linked to an external reservoir whose liquid level was used to set the desired water tension by the suction cell method. The greater the distance between the membrane and the external reservoir, the greater the water tension and the lower the water content (Beuger and Gostomski, 2009). The biofilters were placed inside an insulated, temperature controlled box to maintain the desired operating temperatures.

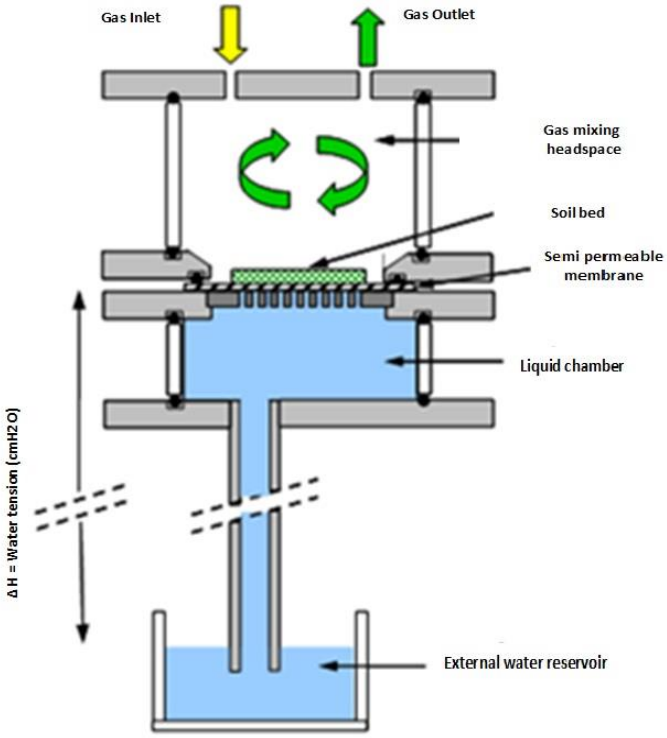



Figure 1: Section view of the differential biofilter with robust water content control.

## 3. ANALYTICAL TECHNIQUES

Samples from the inlet and outlet streams were continuously withdrawn via heat traced (outlet) 1/8$^{th}$ inch stainless steel tubing sampling lines connected to a 10-port valve (Valco) and injected into a GC/FID (SRI-8610C, SRI instruments). Toluene concentration for the reactor inlet and outlets were measured through the GC and simultaneously $CO_2$ was measured out of the GC outlet port during the sample valve purge given for each GC measurements using a $CO_2$ probe (GMP 343, Vaisala). At standard conditions the reactor inlet was measured in quadruplicate once a day and the outlet in quadruplicate thrice a day. Simultaneously outlet $CO_2$ was measured thrice a day. Dry matter content of the soil samples were carried out in triplicate from representative samples for 24 hours at 105 °C before start-up of reactors. Soil samples were cooled in a desiccator for 15 minutes prior to weighing. Total carbon (TC) for both the soil and liquid samples was done by using model 5050 total carbon analyser (Shimazdu).

*Pseudomonas putida* cultures were grown in Luria Bertani (LB) media (Sigma Aldrich) in shake flasks at 40$^{o}$C overnight and late logarithmic phase cultures were added to the membrane covering an active surface area of 0.0043 m$^2$ instead of soil. Microscopy was done on a Leica TCS

SP5 confocal microscope. Wheat germ agglutin-647 was used for differential staining of polysaccharides.

### 3.1. Statistical Analysis

Analysis of variance (ANOVA) was used to evaluate the environmental parameters of temperature, tension and substrate concentrations and interactions using R statistical software for the dependent variable of $CO_2$ mineralization. One sample t-test was used to evaluate experimental uncertainties of the measurements. Statistical analysis was done at an alpha level of 0.05.

## 4. Influence of environmental parameters on $CO_2$ mineralization

$CO_2$ production reflects microbial activity in oxidative biofilm processes [8, 9]. Carbon substrates are mineralized to $CO_2$ and water with a variable fraction going into biomass and other degradation intermediates during biofiltration of waste gases [1]. Three critical environmental parameters were studied in various combinations to study its impact on the $CO_2$ mineralization. Temperature, water content and residual substrate concentration were considered for these studies which were postulated to impact the substrate utilization and carbon endpoints. These parameters were studied at multiple levels in various combinations to track their influence on the fate of degraded toluene (mineralized $CO_2$ and accumulation in the soild/liquid phase). Each biofilter was operated at a certain combination of temperature, water tension and inlet feed. The residual toluene exiting the reactor varied depending on the metabolic activity for each specific operating condition.

The environmental parameters were analysed through a 3X3 and 2x3 factorial approach (three temperatures, three inlet feed concentrations and three water tensions) (Table 1). A two-way ANOVA suggested significant interactions between temperature and substrate concentration, $F(4,151) = 3.01, p= 0.02$. Another two way ANOVA between temperature and tension also showed significant interactions, $F(2,84)= 87, p<0.05$. Thus the simple main effect of each parameter on subsequent levels of the other parameter was further explored to interpret the change on the dependent variable across levels. The subsequent sections in this study analyse the effect of one variable parameter against controlled ones in influencing the $CO_2$ mineralization.

Table 1: Summary of analysis of variance (ANOVA) results for the studied environmental parameters.

| a) | DF | Sum of Squares | Mean Square | F Value | P Value |
|---|---|---|---|---|---|
| Temperature | 2 | 6518 | 3259 | 37 | 5.66e-14 *** |
| Substrate | 2 | 296 | 148 | 1.7 | 0.185 |
| Interaction | 4 | 1045 | 261 | 3 | 0.01998 |
| Residual | 151 | 13093 | 982 | | 0.020 * |
| b) | DF | Sum of Squares | Mean Square | F Value | P Value |
| Tension | 2 | 19295 | 9648 | 566 | <2e-16 *** |
| Temperature | 1 | 2861 | 2861 | 168 | <2e-16 *** |
| Interaction | 2 | 2968 | 1484 | 87 | <2e-16 *** |
| Residual | 84 | 1430 | 17 | | |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

## 4.1 Effect of temperature and substrate concentration

Biofilter runs were studied at 20 °C, 30 °C and 40 °C operated at a controlled water tension of 10 $cm_{H2O}$ and inlet feed concentrations of 75 ppm, 120 ppm and 193 ppm toluene. $CO_2$ mineralization evolved through the time course of the experimental run and varied as a function of specific environmental parameters. Figure 2 presents the simple main effect plots illustrating the variation in steady state $CO_2$ mineralization found over the entire range of temperatures at each feed concentrations.

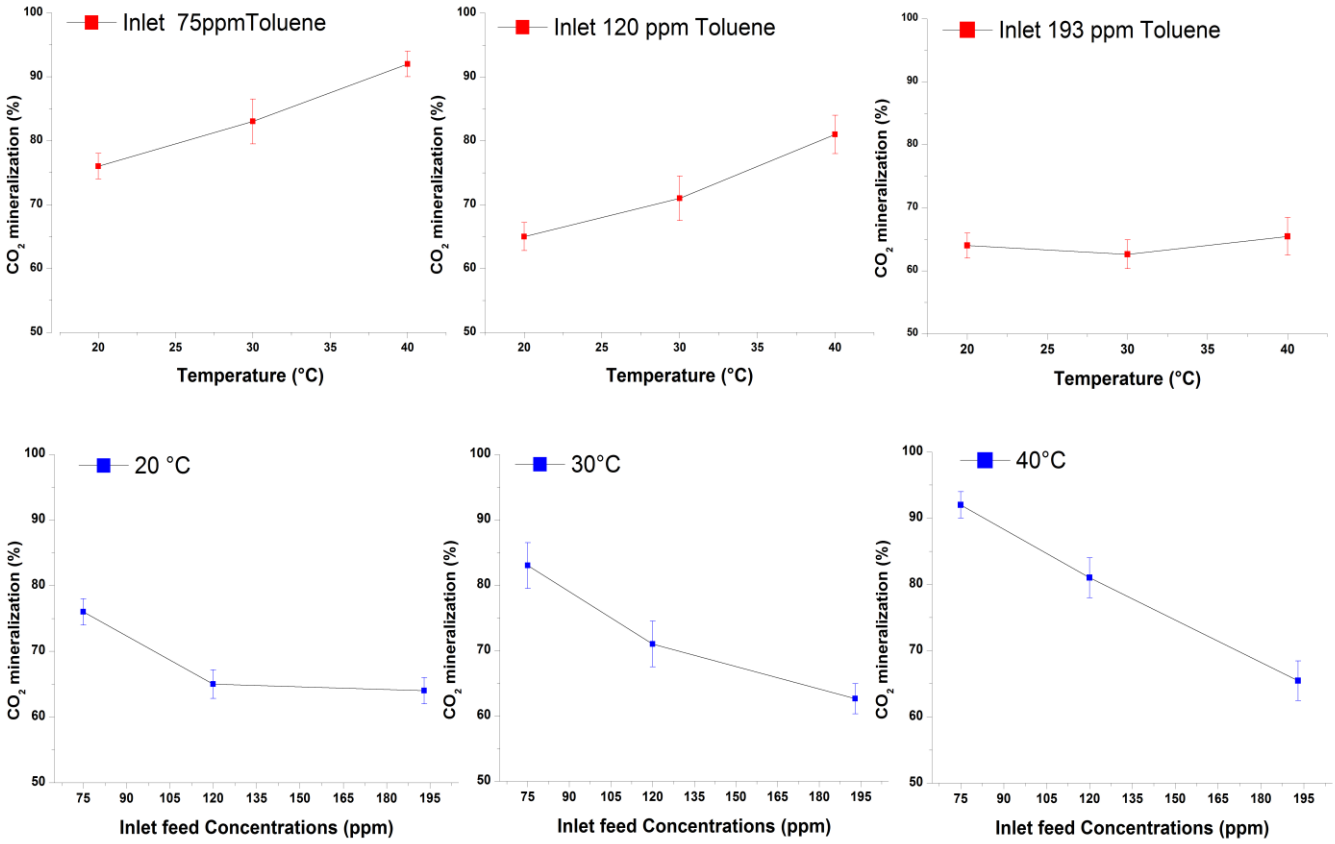



Figure 2 : Simple main effect plots of one parameter against each level of the second parameter. Values represents mean ± standard deviations at 95 % confidence interval (n=10). The uncertainty is the variation in steady state over a 10 day period for each run at given set of conditions.

At a feed concentration of 120 ppm toluene $CO_2$ mineralization increased with temperature (Figure 2). Around 65 % $CO_2$ was mineralized at 20 °C followed by 70 % at 30°C. 40 °C resulted in the highest fraction at 81 % recovery as $CO_2$. The physiology of the toluene degraders could vary across a temperature range [10]. This trend signifies the influence of temperature on substrate utilization patterns. Carbon recovery as $CO_2$ found in this study is in the range of $CO_2$ recoveries (45-88 %) that have been reported in the literature [3, 11-15].The variation in carbon recovery as a function of temperature does imply its underpinning role in influencing metabolic pathways for substrate utilization. To further this

study, the same temperature and water tension conditions were operated at a lower inlet feed concentration of 75 ppm toluene to generate lower residual toluene concentrations. From Figure 2 it can be seen that the $CO_2$ mineralization trend was similar with more of it being mineralized at higher temperatures. At lower inlet toluene concentrations, the fraction of carbon mineralized to $CO_2$ increased. At 40 $^oC$ around 92 % was mineralized to $CO_2$ followed by 82 % at 30 $^oC$ and 70 % at 20 $^oC$. For the third inlet feed of 193 ppm toluene generating higher residual substrate concentrations $CO_2$ mineralization further decreased. However, the mineralized fractions do not differ significantly at ($p<0.05$) as a function of temperature. Thus $CO_2$ mineralization was found to be influenced by the interaction of both the parameters. Significant differences among parameters at each combination were accessed by Tukey's HSD test. Significant differences were found between temperatures at lower substrate concentrations to the ones at higher substrate concentration across various combinations of environmental conditions. The residual toluene concentration and $CO_2$ mineralization in the differential biofilter was also simultaneously impacted by the temperature. Influence of residual toluene concentration on the $CO_2$ mineralization is presented in Figure 3.

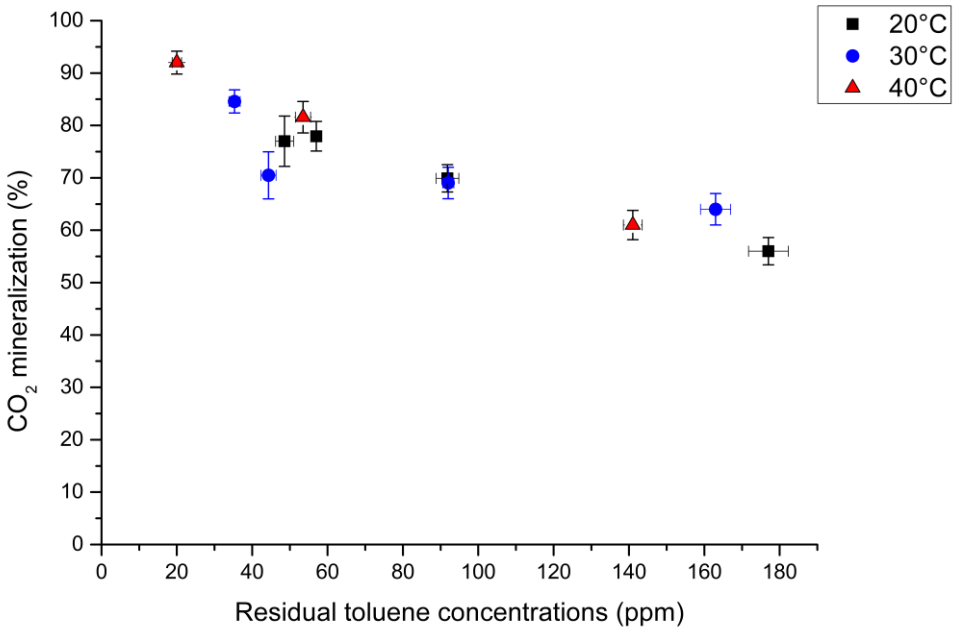



Figure 3: Evolution of residual substrate substrate concentrations across the temperature range for the substrate feed concentrations and its impact of $CO_2$ mineralization. Values represents means ± 95 % confidence interval (n=10) during the steady state period.

From Figure 3 it can be seen that $CO_2$ mineralization decreases with higher residual toluene concentration for all the operating temperatures. Higher temperature (40$^oC$) increased the metabolic activity resulting in lower residual toluene concentrations and simultaneously higher $CO_2$ recoveries were found. An interaction between environmental parameters is likely to drive the process culture's metabolism. Higher residual toluene could also increase the load on the process culture which can lead to variation in degradation products from $CO_2$ mineralization to other non-mineralized fractions. Factors such as substrate toxicity at higher residual concentration could contribute to microbial inactivity [16] apart from influence of lower temperature. Various stress response for microbes have been elucidated which includes production of internal storage polymers like PHA's under nutrient limited conditions [17]. There are also reports of microbes producing more EPS at lower temperatures [18].This phenomenon was reflected by lower $CO_2$ recoveries at higher residual

toluene concentrations at same water tension across the temperature range of (20-40 $^{o}$C) (Figure 3). Less $CO_2$ mineralization is an indication of the culture producing EPS for survival and associated benefits of nutrient pooling [19]. This further illustrates the interdependence and effect of multiple environmental parameters on the fate of degraded carbon in these engineered systems.

### 4.2 Effect of temperature and water tension

Water content is another critical operational parameter influencing biodegradation kinetics [20]. Clearly, as both residual substrate concentration and temperature were interacting to influence the $CO_2$ mineralization, it became imperative to extend the investigation to water tension. Therefore, mineralisation at temperatures of 20 $^{o}$C and 40 $^{o}$C were investigated at two more water tensions of 20 $cm_{H2O}$ and 100 $cm_{H2O}$ at controlled inlet feed of 120 ppm toluene (Figure 4).

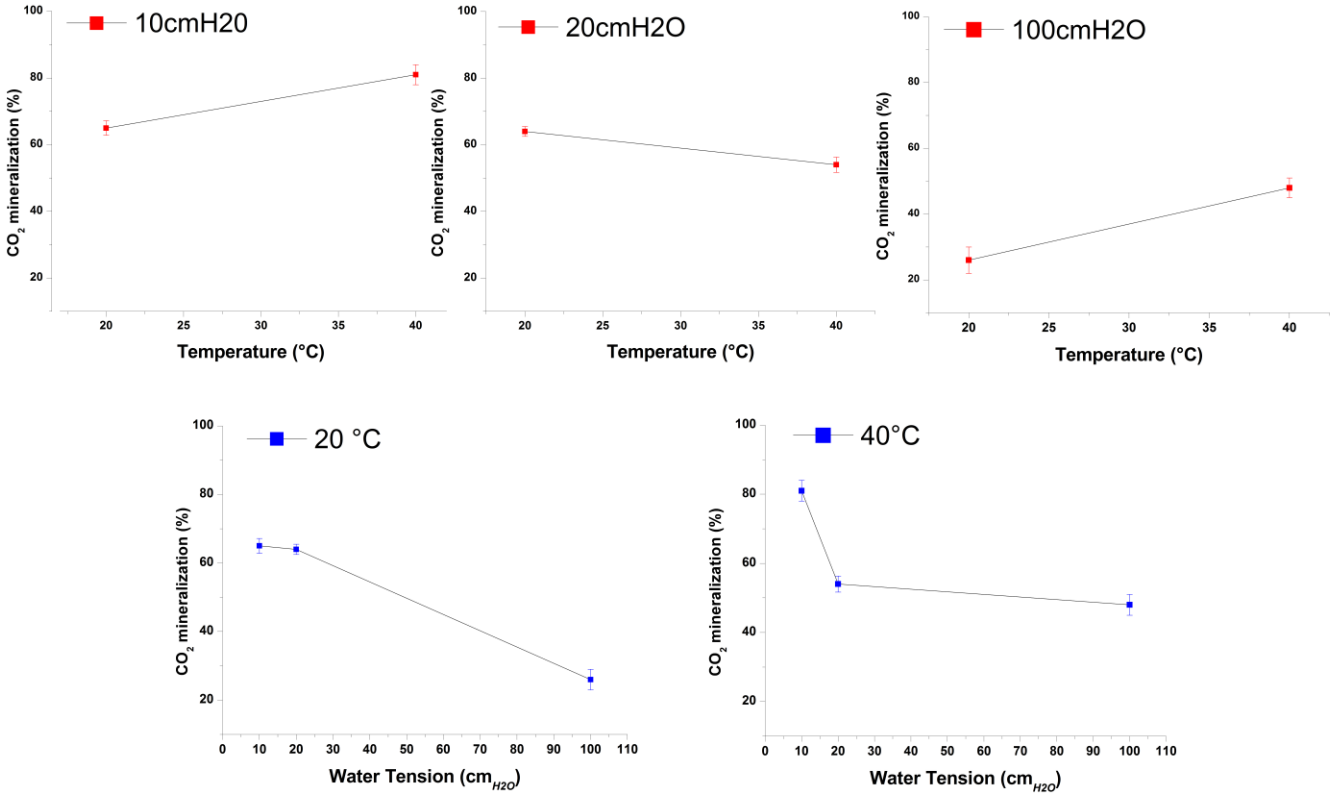



Figure 4: Influence of water tensions at 10 $cm_{H2O}$, 20 $cm_{H2O}$, 100 $cm_{H2O}$ and temperatures on the $CO_2$ mineralization at an inlet concentration of 120 ppm toluene. Values represents mean ± standard deviations at 95 % confidence interval (n=10). The uncertainty is the variation in steady state over a 10 day period for each run at given set of conditions.

From Figure 4 it is apparent that there was significant decrease in mineralisation at 40 $^{o}$C with increase in water tension to 20 $cm_{H2O}$. $CO_2$ mineralization dropped from 81 % to

62 %. On further increasing the water tension to 100 $cm_{H2O}$ $CO_2$ mineralization did not changed significantly. Gallastegui et al. [21] also found only 32% of xylene being recovered as $CO_2$ along with reduced removal efficiency during intermittent water supply leading to low moisture content in the packing media operated at ambient temperature. Gravimetric analysis of the post run biofilter soil revealed soil water content of 0.6 g/g at 10 $cm_{H2O}$ to 0.45 g/g at 20 $cm_{H2O}$ on a dry weight basis. Soil water content was further reduced to 0.33 g/g at 100 $cm_{H2O.}$Clearly there is a departure in $CO_2$ mineralization trend with higher water tension which is evident that can be linked to a shift in substrate utilization mechanisms possibly as a survival mechanism in response to decreasing matric potential [22]. On the contrary there was not much change in $CO_2$ mineralization at 20 $^oC$ from 10 to 20 $cm_{H2O}$ but at 100 $cm_{H2O}$ $CO_2$ mineralization dropped significantly to 25 %. Thus it suggests an intricate interaction between temperature and water tension influencing process metabolism. Further studies at higher water tension at different substrate concentrations should entail more insights into the temperature-tension dynamics on the fate of carbon.

## 5 Biofilter Performance

Biodegradation kinetics can vary significantly with changes in temperature, pollutant concentration and water content. The volumetric toluene degradation rate was quantified by elimination capacity (EC). Figure 5 presents the steady state removal rates achieved over the course of this study at various operational parameters.

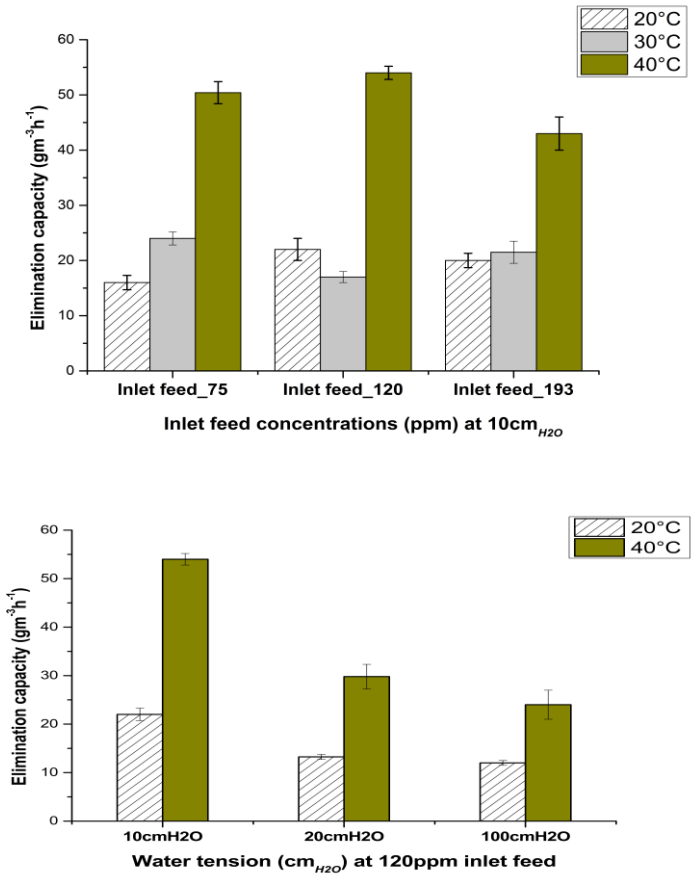



Figure 5: Evolution of elimination capacities (EC) of the biofilters over the steady state period. Values represents mean ± standard deviations at 95 % confidence interval (n=10). The uncertainty is the variation in steady state over a 10-day period for each run at given set of conditions.

From Figure 5 it can be deduced that temperature and tension both had an effect on the degradation rates of the systems. In general, higher temperature resulted in higher EC's independent of other parameters. This can be attributed to the influence of temperature on the metabolic activity of the process culture. The increasing toluene degradation with temperature found in this study compares well to previous studies in the literature [23-25].

Biofilters operated at the same temperature with varying matric tension displayed different degradation capability. Higher degradation was observed at a matric tension of 10 $cm_{H2O}$. At 40 °C the EC dropped from 54 ± 1 $gm^{-3}h^{-1}$ at water tension of 10 $cm_{H2O}$ to 30 ± 2 $gm^{-3}h^{-1}$ at 20 $cm_{H2O}$. The drop in performance at 20 °C followed a similar trend decreasing from 22 ± 1.3 $gm^{-3}h^{-1}$ at water tension of 10 $cm_{H2O}$ to 15 ± 0.5 $gm^{-3}h^{-1}$ at 20 $cm_{H2O}$. At 100 $cm_{H20}$ performance at 20 °C was not affected but operations at 40 °C has a further decrease in EC to 24 ± 3 $gm^{-3}h^{-1}$. Microbes adapt to a decrease in water content through regulation of cellular turgor pressure to prevent dehydration by mobilizing osmolyte concentration [26]. But at lower matric potential, the drier environment can hinder optimal carbon flow effecting osmolyte accumulation [27].These findings on loss in biofilters performance efficacy with water tensions align with earlier studies reporting almost 50% decrease in elimination capacity over a matric potential of -20 to -100 cm $H_2O$ [7]. Similar results were reported by Cabeza *et al.* [28] using compost degrading α-pinene.

## 6 Biofilter with *Pseudomonas putida* biofilm

This experiment was set up with a biofilm of *Pseudomonas putida* isolated from the soil used in the biofilters. The biofilter was operated at a water tension of 10 $cm_{H2O}$ and 40°C with an inlet feed concentrations of 120 ppm toluene with no supplemental nutrient addition. The degraded toluene was tracked in the gas, liquid and solid phase on a carbon basis (Figure 6).

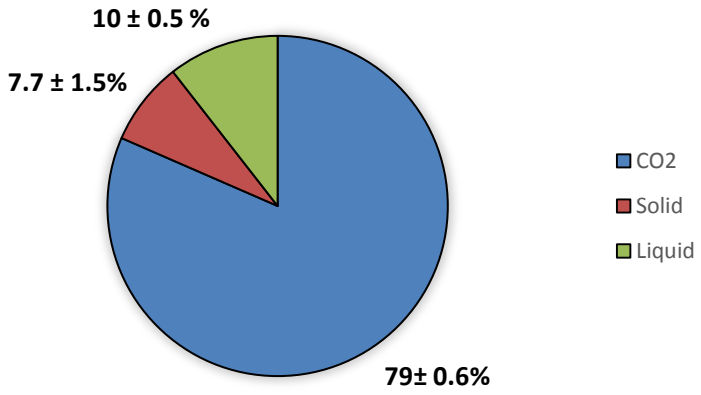


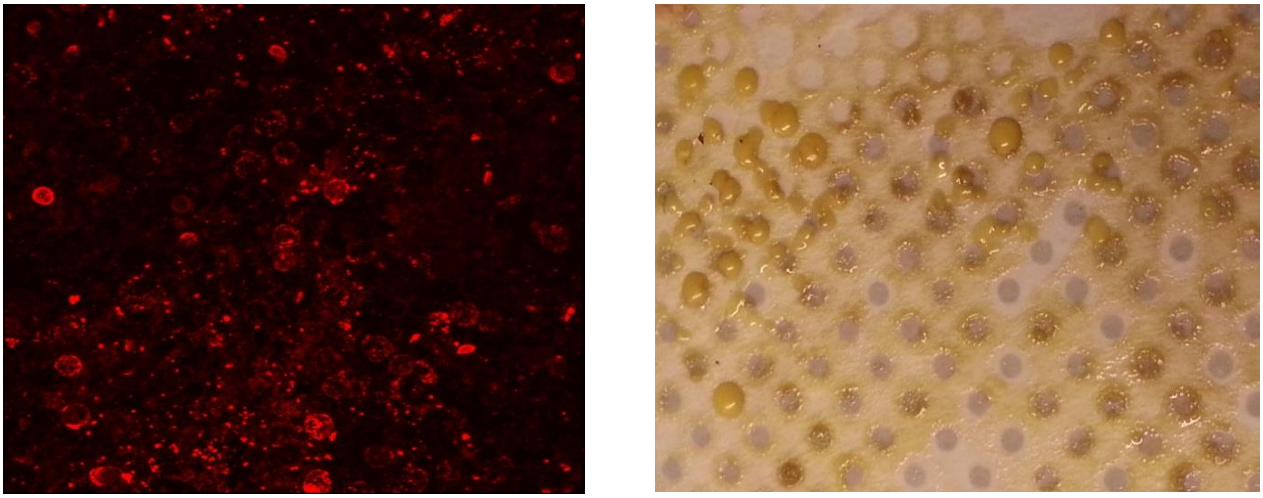

Figure 6: The endpoints of the toluene degraded by Pseudomonas putida reported on a percent carbon basis.

For the biofilm reactor 79 % of the degraded carbon was mineralized to $CO_2$. This is not significantly less compared to 81 % $CO_2$ mineralization for the soil biofilters at similar conditions. Soil biofilters might also have additional secondary degraders in a mixed ecosystem. However since the mineralization was never 100 % for this non-growth system, it indicated the accumulation of the remaining fraction in the solid and liquid phase. Solid and liquid phase accounted for ~18 % of the degraded carbon. The liquid phase in the biofilm was also PBS without any nutrient supplements with negligible carbon content at start-up. The 10 % carbon tracked in the liquid phase is likely soluble microbial products (SMP) from the toluene degradation accumulated over the time course of the experiment.

Likewise a clear biofilm deposition was visualized on the semipermeable membrane upon which the culture was deposited (Figure 7). This was postulated to be extracellular

polysaccharides (EPS) which can be an important carbon sink during nutrient-limited growth [29]. Also EPS has been demonstrated to have a higher C/N ratio than viable biomass [30], thus suggesting negligible nitrogen requirement for providing a carbon sink from toluene oxidation [31]. There was also a possibility of polyhydroxybutyrate (PHB) production at excess residual toluene concentrations. However clearly there would be a limit to the amount of carbon that could be deposited as PHB.

Representative biofilm samples were qualitatively analysed through confocal laser scanning microscopy (CLSM) to identify carbon fractions in the biofilm. Samples were analysed by staining with wheat germ agglutin (WGA-647) which differentially stains polysaccharides [32]. Figure 7 (a) is a 3D stacked confocal microscopy image of a representative post run biofilm sample Figure 7 (b). It clearly implies the presence of polymeric substances in the biofilm as can be visualized from the micrographs. These results corroborates the accumulation of non-mineralized fractions in the solid phase and soluble fractions into the liquid phase as quantified for carbon balance results discussed later. Thus for soil biofilters long-term accumulation of polymeric substances would pose operational problems like clogging leading to pressure drops.



(a) (b)

Figure 7: Differential staining of biofilm samples visualized through CLSM. (a) WGA-647 for polysaccharides (b) Photograph of the biofilm developed on the membrane

## 7 Carbon balance

A key goal of this work was to determine if all the degraded toluene was being accounted for on a carbon basis. Toluene and $CO_2$ were measured continuously and total carbon in the liquid/solid phase was measured at the end of each run. This balance was performed for multiple experiments as a function of various environmental parameters (Table 1). Previous attempts to close the carbon balance have been uneven, with often 10-50 % of the degraded reported carbon missing [3, 23, 33-36]. Overall carbon balances for this study averaged 111% ± 9% with the total carbon of the solid phase contributing the most to the uncertainty (6-8%). The mean quantitative carbon balance did not differ significantly from the theoretical mean of 100% mass balance closure with $t(9)= 1.75$, $p=0.11$. Therefore we are confident that we are tracking all the carbon endpoints for all the toluene being degraded.

## 7 Conclusion

The fate of the degraded toluene has been tracked conclusively over the experimental runs in soil. Investigations on multiple parameters identified trends on $CO_2$ mineralization and the eventual fate of carbon in the system. Tracking the $CO_2$ mineralization fraction as a function of various environmental parameters provided interesting insights on their interactions pertaining to elimination capacities and variations in carbon-endpoints. Further, *Pseudomonas putida* biofilm experiments at similar conditions confirmed the carbon end-points of the non-mineralized toluene as polysaccharides through microscopic studies. Thus a better understanding of the degradation product ratios could immensely help to optimize both process efficacy and address operational problems like pressure drop owing to excessive clogging.

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**REFERENCES**


1. Devinny, J.S., M.A. Deshusses, and T.S. Webster, *Biofiltration for air pollution control*. 1999: CRC.
2. Iranpour, R., et al., *Literature review of air pollution control biofilters and biotrickling filters for odor and volatile organic compound removal.* Environmental Progress, 2005. **24**(3): p. 254-267.
3. Deshusses, M.A., *Transient Behavior of Biofilters: Start-Up, Carbon Balances, and Interactions between Pollutants.* Journal of Environmental Engineering, 1997. **123**(6): p. 563-568.
4. Jorio, H., R. Brzezinski, and M. Heitz, *A novel procedure for the measurement of the kinetics of styrene biodegradation in a biofilter.* Journal of Chemical Technology and Biotechnology, 2005. **80**(7): p. 796 -804.
5. Grove, J.A., et al., *Estimation of carbon recovery and biomass yield in the biofiltration of octane.* Environmental Engineering Science, 2009. **26**(10): p. 1497 -1502.
6. Wang, C., X. Kong, and X.-Y. Zhang, *Mesophilic and thermophilic biofiltration of gaseous toluene in a long-term operation: Performance evaluation, biomass accumulation, mass balance analysis and isolation identification.* Journal of Hazardous Materials, 2012. **229 - 230**(0): p. 94 -99.
7. Beuger, A.L. and P.A. Gostomski, *Development of a biofilter with water content control for research purposes.* Chemical Engineering Journal, 2009. **151**(1 - 3): p. 89 -96.
8. Elmrini, H., et al., *Biofiltration of xylene emissions: bioreactor response to variations in the pollutant inlet concentration and gas flow rate.* Chemical Engineering Journal, 2004. **100**(1 -3): p. 149 -158.
9. Kroukamp, O. and G.M. Wolfaardt, *CO2 production as an indicator of biofilm metabolism.* Applied and Environmental Microbiology, 2009. **75**(13): p. 4391 -4397.
10. Stewart, P.S. and M.J. Franklin, *Physiological heterogeneity in biofilms.* Nature Reviews Microbiology, 2008. **6**(3): p. 199 -210.
11. Fürer, C. and M.A. Deshusses. *Biodegradation in Biofilters: Did the Microbe Inhale the VOC?, in Proceeding of the A&WMA 93rd Annual Meeting. 2000: Salt Lake City, Utah. p. 13.* 2000.
12. Jorio, H., L. Bibeau, and M. Heitz, *Biofiltration of Air Contaminated by Styrene: Effect of Nitrogen Supply, Gas Flow Rate, and Inlet Concentration.* Environmental Science & Technology, 2000. **34**(9): p. 1764 -1771.
13. Li, G.W., et al., *Use of Biological Activated Carbon to Treat Mixed Gas of Toluene and Benzene in Biofilter.* Environmental Technology, 2002. **23**(4): p. 467 -477.

14. Xi, J., H.-Y. Hu, and Y. Qian, *Effect of operating conditions on long-term performance of a biofilter treating gaseous toluene: Biomass accumulation and stable-run time estimation.* Biochemical Engineering Journal, 2006. **31**(2): p. 165 -172.
15. Bester, E., et al., *Biofilm form and function: carbon availability affects biofilm architecture, metabolic activity and planktonic cell yield.* Journal of Applied Microbiology, 2011. **110**(2): p. 387 -398.
16. Song, J. and K.A. Kinney, *Microbial response and elimination capacity in biofilters subjected to high toluene loadings.* Applied Microbiology and Biotechnology, 2005. **68**(4) : p. 554-559.
17. Poblete-Castro, I., et al., *The metabolic response of P. putida KT2442 producing high levels of polyhydroxyalkanoate under single-and multiple-nutrient-limited growth: Highlights from a multi-level omics approach.* Microb Cell Fact, 2012. **11**(1): p. 34.
18. Le Bihan, Y. and P. Lessard, *Monitoring biofilter clogging: biochemical characteristics of the biomass.* Water Research, 2000. **34**(17): p. 4284 -4294.
19. Xavier, J.B. and K.R. Foster, *Cooperation and conflict in microbial biofilms.* Proceedings of the National Academy of Sciences, 2007. **104**(3): p. 876 -881.
20. Delhoménie, M.-C. and M. Heitz, *Biofiltration of air: a review.* Critical Reviews in Biotechnology, 2005. **25**(1 -2): p. 53 -72.
21. Gallastegui, G., et al., *Evaluating the impact of water supply strategies on p-xylene biodegradation performance in an organic media-based biofilter.* Journal of Hazardous Materials, 2011. **185**(2 ‐ 3): p. 1019 -1026.
22. Schimel, J., T.C. Balser, and M. Wallenstein, *Microbial stress-response physiology and its implications for ecosystem function.* Ecology, 2007. **88**(6): p. 1386 -1394.
23. Cox, H.H.J., et al., *Thermophilic biotrickling filtration of ethanol vapors.* Environmental Science & Technology, 2001. **35**(12): p. 2612 -2619.
24. Mohammad, B.T., M.C. Veiga, and C. Kennes, *Mesophilic and thermophilic biotreatment of BTEX-polluted air in reactors.* Biotechnology and Bioengineering, 2007. **97**(6): p. 1423 -1438.
25. Jin, Y., et al., *Fungal biofiltration of α ‚pinene: Effects of temperature, relative humidity, and transient loads.* Biotechnology and Bioengineering, 2007. **96**(3): p. 433 -443.
26. Van De Mortel, M. and L.J. Halverson, *Cell envelope components contributing to biofilm growth and survival of Pseudomonas putida in low ‚water ‚content habitats.* Molecular microbiology, 2004. **52**(3): p. 735 -750.
27. Kakumanu, M.L. and M.A. Williams, *Osmolyte dynamics and microbial communities vary in response to osmotic more than matric water deficit gradients in two soils.* Soil Biology and Biochemistry, 2014. **79**: p. 14-24.

28. Cabeza, I., et al., *Biofiltration of α-pinene vapours using municipal solid waste (MSW) ' Pruning residues (P) composts as packing materials.* Chemical Engineering Journal, 2013. **233**: p. 149-158.
29. Weber, F.J. and S. Hartmans, *Prevention of clogging in a biological trickle-bed reactor removing toluene from contaminated air.* Biotechnology and Bioengineering, 1996. **50**(1): p. 91 -7.
30. Leddy, M.B., D.W. Phipps, and H.F. Ridgway, *Catabolite-mediated mutations in alternate toluene degradative pathways in Pseudomonas putida.* Journal of Bacteriology, 1995. **177**(16): p. 4713 -4720.
31. Wilshusen, J.H., et al., *Methane oxidation and formation of EPS in compost: effect of oxygen concentration.* Environmental Pollution, 2004. **129**(2): p. 305 -314.
32. Strathmann, M., J. Wingender, and H.-C. Flemming, *Application of fluorescently labelled lectins for the visualization and biochemical characterization of polysaccharides in biofilms of Pseudomonas aeruginosa.* Journal of Microbiological Methods, 2002. **50**(3) : p. 237-248.
33. Avalos Ramirez, A., et al., *Treatment of methanol vapours in biofilters packed with inert materials.* Journal of Chemical Technology & Biotechnology, 2008. **83**(9): p. 1288 -1297.
34. Song, J. and K.A. Kinney, *Effect of vapor-phase bioreactor operation on biomass accumulation, distribution, and activity: Linking biofilm properties to bioreactor performance.* Biotechnology and Bioengineering, 2000. **68**(5): p. 508 -516.
35. Girard, M., et al., *Biofiltration of methane at low concentrations representative of the piggery industry ⁄ Influence of the methane and nitrogen concentrations.* Chemical Engineering Journal, 2011. **168**(1): p. 151 -158.
36. Morales, M., S. Revah, and R. Auria, *Start ¸up and the effect of gaseous ammonia additions on a biofilter for the elimination of toluene vapors.* Biotechnology and Bioengineering, 1998. **60**(4): p. 483 -491.