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RESEARCH PAPER

Studies on the Influence of Different Metabolic Uncouplers on the Biodegradation of Toluene in a Differential Biofilter Reactor

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Abstract One of the biggest challenges in a traditional biofilter is to overcome the low volumetric degradation rate, which often makes the footprint excessive. The volumetric degradation rate or elimination capacity (EC) is directly influenced by the specific biodegradation rate of the microorganisms involved in degrading the pollutants. Application of metabolic uncouplers in biofiltration could improve the biodegradation rate of microorganisms, which in turn could increase the EC. The addition of metabolic uncouplers to the growth system decreases the biomass growth whereas in energy-excess, non-growth systems like biofiltration, it is expected to increase the specific substrate uptake rate since the substrate requirement for maintenance energy should increase, which in turn should increase the EC. Seven potential metabolic uncouplers were screened in batch serum bottles and subsequently tested in a continuous biofilter reactor with soil as the biofilter medium. The metabolic uncouplers tested were benzoic acid, carbonylcyanide p-trifluoromethoxy phenylhydrazone (FCCP), carbonylcyanide m-chloromethoxy phenylhydrazone (CCCP), pentachlorophenol (PCP), malonic acid, 2, 4, 6-trichlorophenol (TCP) and m-chlorophenol (mCP). Only PCP and 2, 4, 6-TCP increased the toluene degradation rate significantly. PCP increased the toluene degradation rate by 35% at 140 μM, whereas 4051 μM TCP increased the rate by 18%. FCCP did not significantly affect the degradation rate and the other metabolic uncouplers decreased the degradation rate.

Keywords: VOC, biofilter, metabolic uncoupler, toluene, pentachlorophenol, trichlorophenol

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1. Introduction

Biological control of air pollution has many operational and cost advantages over conventional physico-chemical methods in many industrial applications. Biofiltration is an air pollution control technology frequently used for treating odour and volatile organic compounds from waste air streams. It is a cost-effective approach to volatile organic compound (*e.g.* toluene) removal for large air flows ($> 1{,}000\ m^3/h$ and low concentrations $< 1{,}000$ ppm) [1]. Under optimum conditions, microorganisms present in a biofilter medium convert the absorbed biodegradable contaminants into carbon dioxide, salt and water [2-5]. Although biofiltration is a simple and environmentally friendly technology, one of the biggest challenges to overcome is the low volumetric degradation rate in traditional biofilters, which often makes the footprint excessive.

Elimination capacity (EC), the volumetric degradation rate ($g/m^3/h$) is directly influenced by the biodegradation rate of the microorganisms involved in degrading the pollutant. Application of metabolic uncouplers in biofiltration could improve the biodegradation rate of microorganisms indirectly, which in turn could increase the EC. The inability of chemiosmotic mechanisms associated with electron transfer to generate the theoretical amount of metabolic energy is termed metabolic uncoupling [6]. Chemical species which have the potential to induce this metabolic uncoupling are called metabolic uncouplers [7]. They were originally used to elucidate energy production in mitochondria and more

recently to lower sludge production in waste water treatment and disrupt biofilm formation [8-10]. The addition of metabolic uncouplers to the growth system decreases the biomass growth whereas in energy-excess, non-growth systems like a biofilter, it is expected to increase the specific substrate uptake rate since the substrate demand for maintenance increases and in turn should increase the EC.

The objective of this work is to compare the effectiveness of seven metabolic uncouplers in increasing the EC and to determine their optimal concentration in the biodegradation of air-borne toluene by soil. To accomplish this, a differential biofilter reactor with rigorous water content control developed by Beuger and Gostomski (2009) is used in our current study.

2. Materials and Methods

Experiments for studying the effect of metabolic uncouplers in batch mode used serum bottles and a Varian-3800 gas chromatography system (Agilent Technologies, USA). Experiments carried out to study the effect of seven potential metabolic uncouplers in continuous mode used three differential biofilter reactors (Reactor 2, Reactor 3 and Reactor 4) of similar configurations (Fig. 1) developed by Beuger and Gostomski (2009) with online sample and carbon dioxide monitoring system [11,12]. The reactors were autoclaved prior to assembling at 121°C for 45 min in order to prevent biological growth in the water reservoirs. Toluene laden air was supplied to the reactors through

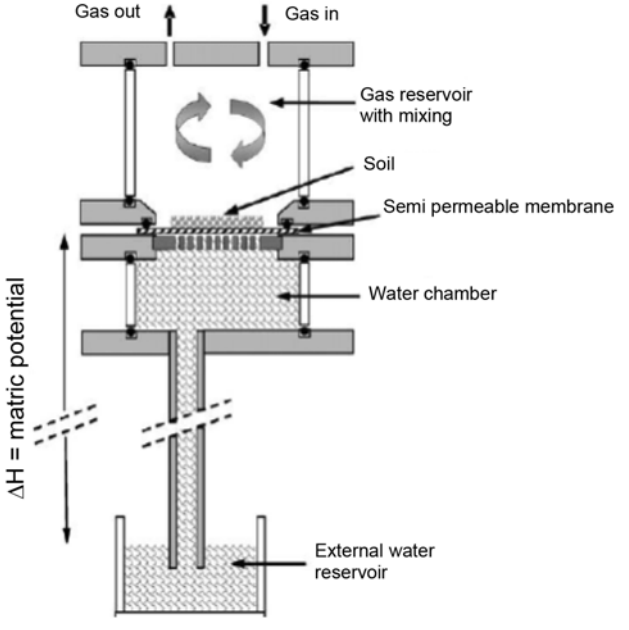

Fig. 1. A cut-away section of the differential biofilter reactor with water content control.

mass flow controllers (MKS Instruments, USA) and a diffusion flask/water bath system at 25 ml/min throughout the experiment. It was humidified with a shell-in-tube humidifier system (Perma Pure LLC, USA) before entering the reactor. Each reactor and humidifier was placed in a temperature controlled box at 30°C. A cooling load was applied to the box by a refrigeration unit (Thermoelectric Refrigeration Ltd., New Zealand) to control the reactor temperature when the room temperature was high. Approximately 8.65 g wet weight of sieved soil (no.6 mesh) was loaded on the mixed cellulose ester membrane (0.45 μm pore size and 90 mm diameter) using a stainless steel ring of 53 mm ID and 3 mm depth and the reactor was sealed. However, to confirm no abiotic toluene losses, all the reactors were operated without soil initially and, after confirming there was no toluene loss following the initial equilibrium between the air phase and water reservoir, soil was loaded into all reactors. A matric potential of -10 cm was achieved by placing and sealing the external water reservoir below the membrane. The suction cell set-up used to control and manipulate the water content in the soil bed was also used effectively to supply and control the metabolic uncoupler addition to the soil inside the reactors during the screening studies. Toluene concentrations at the reactor inlet and outlet were measured by an integrated online gas chromatography system (SRI Instruments, USA). The outlet sample line from the reactor was heat traced at 40°C until GC entry point to prevent water condensation. A phosphate buffered saline (PBS) solution [13] was used to prepare the required concentration of metabolic uncouplers used in both batch and continuous screening studies. The solution pH was adjusted to 7.0 and then autoclaved. Since most of these metabolic uncouplers were sparingly soluble (also non-volatile) in water at room temperature, autoclaving the solution at 121°C helped to dissolve the metabolic uncouplers and as well in preparing an abiotic solution for the screening experiments.

2.1. Screening of metabolic uncouplers in batch mode

Nine metabolic uncouplers (Table 1) were chosen for the initial short-term screening tests their ability to increase the toluene degradation rate by soil microorganisms. Since no research work has been done in biofiltration with the application of metabolic uncouplers, the nine metabolic uncouplers (Table 1) which were earlier reported in growth systems like activated sludge process have been selected for the current study. Hence, the selection of these metabolic uncouplers was purely on an assumption that these metabolic uncouplers would have an influence on a non-growth system like ours based on their previous reports on growth systems. Approximately 8.65 g wet weight of soil (Park House Garden Supplies, Christchurch, New Zealand)

Table 1. Metabolic uncouplers used in batch mode serum bottle tests

Metabolic uncoupler	Effective conc. reported (μM)	Concentration tested (μM)	Solubility [14] (mM)	pKa
benzoic acid (BA)	10,000 [15]	10,000	23.8	4.20 [16]
carbonylcyanide m-chloromethoxy-phenylhydrazone (CCCP)	10 [17]	0.01	NA	6.09 [18]
carbonylcyanide p-trifluoromethoxy-phenylhydrazone (FCCP)	10 [17]	0.01	NA	6.10 [18]
2,4-dinitrophenol (DNP)	760 [19]	76	7.6	4.09 [20]
m-chlorophenol (mCP)	160 [21]	160	22.6	8.80 [22]
malonic acid (MA)	96 [23]	96	701.5	2.83 [24]
p-nitrophenol (pNP)	860 [22]	860	107.8	7.15 [20]
Pentachlorophenol (PCP)	142 [25]	142	0.15	4.70 [26]
2,4,6-trichlorophenol (TCP)	10 [27]	10	4.05	7.50 [28]

Fig. 2. Batch mode serum bottle experimental set-up (inside a 30°C incubator).

was placed on a Whatman filter paper (500 mm dia., Grade 1) in a funnel over a flask. A 0.01 M calcium chloride (Used in-order to maintain hardness/compactness of the soil) solution was then used to make up 100 mL of metabolic uncoupler solutions at the concentration reported in the literature for activated sludge studies. However, based on earlier experiments, the concentration of dinitrophenol, carbonylcyanide m-chloromethoxy-phenylhydrazone (CCCP) and carbonylcyanide p-trifluoromethoxy-phenylhydrazone (FCCP) were decreased to 10% of the literature values for this screening study. The metabolic uncoupler solution was poured over the soil and then the soil sample was squeezed to remove any excess solution. The wet soil (20% (wet weight) water content was measured in the wet soil) treated with metabolic uncoupler was then transferred into a 125 mL serum bottle. Approximately 0.5 μL of HPLC (High Performance Liquid Chromatography) grade toluene was then injected into the serum bottle to generate approximately 700 ppm of toluene vapour in the head space sealed with a stopper and a cap. Four control serum bottles were also used in this study with no soil, abiotic soil, soil without any metabolic uncoupler and soil with 2 mL of toluene degraders (mixed culture). The toluene degraders used in the control study were previously isolated from soil and mixed together to form the mixed culture (*Pseudomonas putida, Stenotrophomonas maltophilia*, *Pseudomonas citronellolis* and *Ochrobactrum tritici*- Confirmed using 16S and 18S rDNA analysis by M/s. Eco Gene Ltd., New Zealand). Each condition was tested in duplicate. All the serum bottles were incubated at 30°C for 60 h (Fig. 2). Periodically, toluene samples from the serum bottles were analysed by gas chromatography system to observe the degradation.

2.2. Screening of metabolic uncouplers in continuous mode

Following the batch screening studies, seven metabolic uncouplers (Residual metabolic uncouplers were removed through multiple PBS washes at the end of each experiments) were used for further screening studies in the continuous differential biofilter reactors (Table 2). Based on our earlier studies carried out on nutrient limitation (data not shown), preference was given to the non-nitrogen containing metabolic

Table 2. Metabolic uncouplers used in continuous mode screening test

Metabolic uncoupler	Concentrations tested (μM)
benzoic acid (BA)	5,000, 10,000, and 15,000
pentachlorophenol (PCP)	70 and 140
2,4,6 trichlorophenol (TCP)	4,051
malonic acid (MA)	25, 50, and 100
carbonylcyanide p-chloromethoxy phenylhydrazone (CCCP)	1, 2, and 10
carbonyl cyanide p-trifluoromethoxy-phenylhydrazone (FCCP)	10 μM
m-chlorophenol (mCP)	16, 160, and 1,600

uncouplers for testing in continuous mode. Initially, all the biofilter reactors ran without metabolic uncouplers until a steady toluene degradation rate was observed following the introduction of soil inside the reactors. Following each metabolic uncoupler concentration tested, fresh PBS was used to remove the residual metabolic uncoupler in the soil until a steady toluene degradation rate was observed. The average inlet toluene concentration used in all three biofilter reactors was approximately 180 ppm. Each metabolic uncoupler experiment was carried out for more than 30 days at all uncoupler concentrations following the initial steady state EC (before the addition of metabolic uncoupler) in each of the three differential biofilter reactors. Following experimentation, the tested liquid samples (PCP and TCP) were sent for analysis at Hill Laboratories, New Zealand for PCP and TCP analysis.

3. Results and Discussion

3.1. Batch mode screening test

The effect of nine metabolic uncouplers on toluene degradation in serum bottle tests is shown in Fig. 3. It was observed that in 60 h period, pentachlorophenol, benzoic acid, p-nitrophenol, 2, 4, 6 trichlorophenol and m-chlorophenol increased the toluene degradation rate by 40% compared to the control soil with toluene degraders and 200% compared to the control soil without toluene degraders. CCCP had a better toluene degradation rate when compared with the control soil without toluene degraders. The addition of the uncouplers 2,4 dinitrophenol, malonic acid and FCCP decreased the toluene degradation rate when compared with the control soil with and without toluene degraders. On the basis of batch screening test results, pentachlorophenol, benzoic acid, 2, 4, 6 trichlorophenol, m-chlorophenol and CCCP were selected for further screening studies in a continuous biofilter reactor system. Later, it was decided to test both malonic acid and FCCP also in the continuous biofilter reactor system to understand their degradation dynamics.

It was possible that the soils in the short-term serum bottle tests were not growth limited during the 60 h test period as there may have been residual nutrients in the soil which the microbes might have utilized. A similar response (higher EC) was also observed in the continuous biofiltration reactor during initial acclimation time (data not shown). Hence the toluene degradation might have been mostly due to growth (plus uncoupling) and not due to maintenance requirements enhanced by uncouplers. Hence seven out of nine metabolic uncouplers were selected for further screening studies in continuous biofilter reactor system. However, p-nitrophenol and 2, 4 dinitrophenol were not selected for further studies in the continuous biofilter reactor system as both of them contained nitrogen and our earlier work (data not shown) demonstrated the toluene degraders in the soil were nitrogen limited after the acclimation period.

3.2. Continuous mode screening test

3.2.1. Effect of benzoic acid (BA)

Reactor 3 was used for this study and was run initially for 22 days with fresh soil and PBS which generated a steady state EC of 38.9 g/m^3/h (Fig. 4). Following the steady state response, PBS was replaced with 5,000 μM benzoic acid buffered at pH 7 on the 22nd day. Following steady state

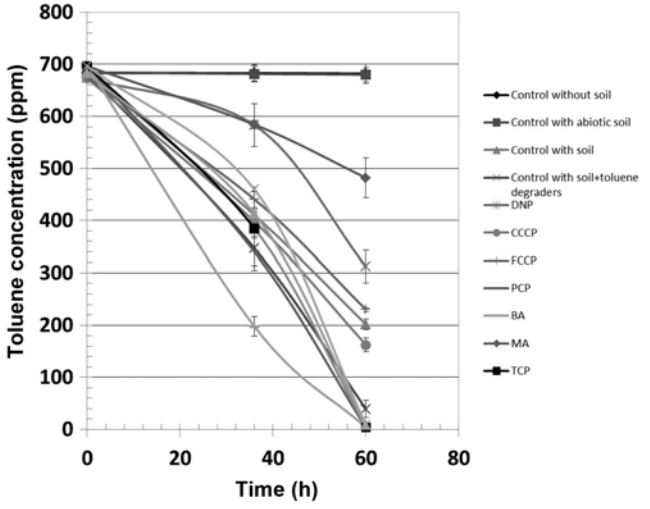

Fig. 3. Effect of different metabolic uncouplers on toluene degradation rate in batch serum bottle tests with soil. Individual error bars are the standard deviation between the duplicates.

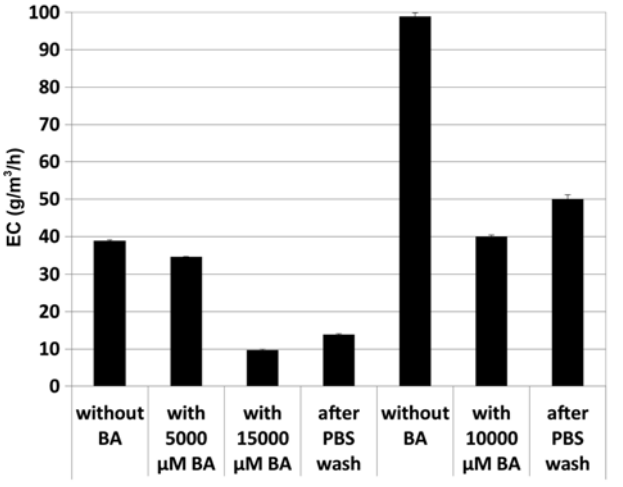

Fig. 4. Effect of benzoic acid on toluene degradation rate in differential biofilter reactor with soil. Error bars are the standard deviation between the multiple sample injections in GC.

EC, it was observed that addition of 5,000 μM benzoic acid decreased the EC by 10.8% with reference to the initial EC. On 25th day, 5,000 μM benzoic acid solution was replaced with a 15,000 μM benzoic acid solution in order to understand the effect of a higher benzoic acid concentration. However, the EC dropped significantly (9.7 g/m^3/h) following the addition of 15,000 μM benzoic acid. Following this response, the 15,000 μM benzoic acid solution was removed from the reactor on 28th day through multiple PBS washes. Despite the PBS washes, the EC recovered to 36% of the initial EC (Fig. 4). This response was not consistent with an uncoupler response as per the chemiosmotic theory. The experiment was repeated with a fresh soil and PBS. After a steady state EC of 99 g/m^3/h, a 10,000 μM benzoic acid solution was added to the reactor replacing PBS. However, a similar trend compared to the earlier experiment was observed. The EC decreased by 60% when compared to the initial EC and after the PBS wash, it recovered only by 10% (Fig. 4).

The benzoic acid concentration reported to be effective in influencing the metabolic fluxes of yeast was 10,000 μM [14]. However, the concentrations tested in the non-growth differential biofiltration system ranged between 5,000 and 15,000 μM. But none of the concentrations tested in the system increased the EC consistent with a metabolic uncoupler rather it decreased the EC. This suggested that benzoic acid might have killed the toluene degraders which are mostly bacteria. Hence the response of benzoic acid in a eukaryotic growth system is dissimilar to a prokaryotic non-growth system. However, reducing the concentration of benzoic acid further below may lead to the possible degradation of benzoic acid by toluene degraders [15,16]. Hence, experiments at lower benzoic acid concentrations were not performed in the current study. Therefore, benzoic acid was considered ill-suited as a potential metabolic uncoupler for enhancing the toluene degradation rate in non-growth biofilter reactor system. The response of benzoic acid was found different in batch and continuous systems. This is because in batch mode benzoic acid was added in non-steady state condition (steady state in continuous mode), which means that though the soil contained toluene degraders; it might also contain non-toluene degraders which might have degraded the benzene.

3.2.2. Effect of pentachlorophenol (PCP)

Reactor 2 was used for this study and was run initially for 29 days with fresh soil and PBS, which generated an initial steady state EC of 71.4 g/m^3/h (Fig. 5). PBS was replaced with a 70 μM PCP solution on the 29th day. The PCP a slowly increased the EC and a steady EC of 84 g/m^3/h was observed after the 33rd day. Following this, a 140 μM PCP solution replaced the 70 μM PCP solution on the 34th day.

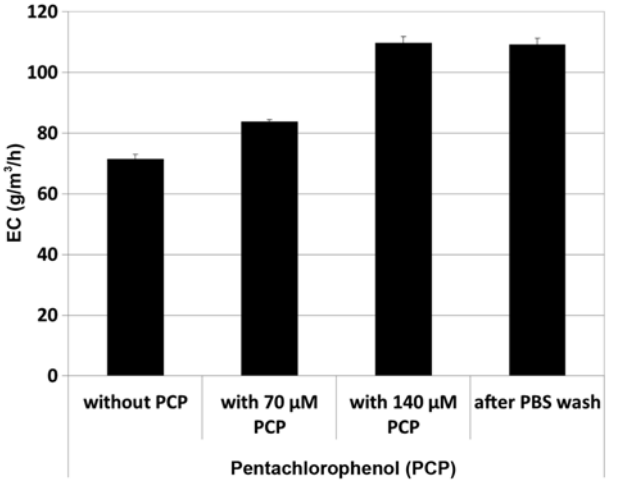

Fig. 5. Effect of pentachlorophenol on toluene degradation rate in differential biofilter reactor with soil. Error bars are the standard deviation between the multiple sample injections in GC.

It was observed that 140 μM PCP increased the EC by 35% when compared with the initial EC. A steady EC of 110 g/m^3/h was observed after 50 days (Fig. 5). Due to the solubility limit of PCP (which is ~150 μM) studies at higher concentrations were not performed. Since PCP was not easily degradable by the soil microbes [17] and that the system was nitrogen-limited, it was concluded that the increase in EC was not directly associated with growth on PCP. A PBS wash to remove the PCP from the reactor produced an EC of 109 g/m^3/h and it did not drop back to the initial EC value. This response was not as expected for uncoupling, as the ATP production efficiency should have returned to the initial level, thereby dropping the EC to its original level.

At least two possibilities existed to explain these results in addition to some level of metabolic uncoupling:

i) the PCP killed microorganisms not associated with toluene degradation thereby freeing up nitrogen for the toluene degraders to grow, thus permanently increasing the EC;
ii) the PCP was not completely removed by the wash step due its hydrophobic property leaving it entrained in the lipid layer of the biomass and adsorbed to the soil;

Following the 140 μM PCP studies, the liquid was sent for PCP analysis (including the PBS washes). The results showed that only 18.4% (Table 3) of PCP did not end up in the removed liquid and subsequent PBS washes. This 18% loss of PCP may be attributed to the PBS wash not removing all PCP from the system. It also confirms that PCP was not significantly degraded by the toluene degraders. If uncoupling was happening, it is expected to be reversible with a return to the initial EC upon removal [8,18]. These

Table 3. Results of PCP analysis in the liquid sample (Source: The Hill Laboratories, NZ)

Metabolic uncoupler	Quantity tested in differential biofiltration reactor (g)	Quantity analyzed /reported from the liquid sample by "The Hill Laboratories, NZ" (g)	% change
Pentachlorophenol	0.038	0.031	18.4%

Table 4. Results of TCP analysis in the liquid sample (Source: The Hill Laboratories, NZ)

Metabolic uncoupler	Quantity tested in differential biofiltration reactor (g)	Quantity analyzed /reported from the liquid sample by "The Hill Laboratories, NZ" (g)	% Change
2,4,6 trichlorophenol	0.79	0.25	68.4%

results imply that growth by the toluene degraders on nitrogen released by other organism probably increased the EC and PCP did not uncouple the metabolism. However, additional experiments analysing the PCP fraction in the soil and with pure cultures of toluene degraders (biofilm) will help further clarify this response.

3.2.3. Effect of 2, 4, 6 trichlorophenol (TCP)

Reactor 3 was used for this study and was run initially for 13 days until it reached a steady EC of 73 g/m^3/h (Fig. 6). Based on the earlier experiment done with PCP, it was decided to use a higher concentration (4,051 µM) of the similar but more soluble TCP to observe the EC change. At 4,051 µM of TCP (which is its solubility limit), the EC increased by 18% when compared with the initial EC. However, similar to PCP, after removing the TCP from the reactor and washing with PBS, the EC did not return to the initial EC and dropped only by 2% when compared with the maximum EC generated by 4,051 µM TCP (Fig. 6). The possible explanations for this response are the same as those for PCP. Similar to PCP studies, TCP was also sent for analysis (after multiple PBS washes). The results showed that 68% (Table 4) of the TCP was missing from the liquid. This particular result raised a question about the possibilities of TCP degradation (either by toluene degraders or by any other microbe present in the soil or in combination of both), as the increased solubility should have aided its removal by washing. In addition, growth on nitrogen released by non-toluene degraders is still a possibility for the increased EC. Similar to PCP, it was decided to test TCP in a pure culture of toluene degrader (biofilm) in our differential biofilter reactor system in order to further understand the potential uncoupling mechanism of TCP clearly.

3.2.4. Effect of malonic acid (MA)

Reactor 2 was used for this study. It was run for nearly 35 days with fresh soil and PBS to get a steady state EC of 109 g/m^3/h (Fig. 7). Three different concentrations of malonic acid were tested in this system with increasing concentration. Increasing concentrations of malonic acid decreased the EC. Initial addition of 25 µM malonic acid buffered at pH 7 on 36th day decreased the EC by 5.8%. Following the steady state EC, 25 µM malonic acid was

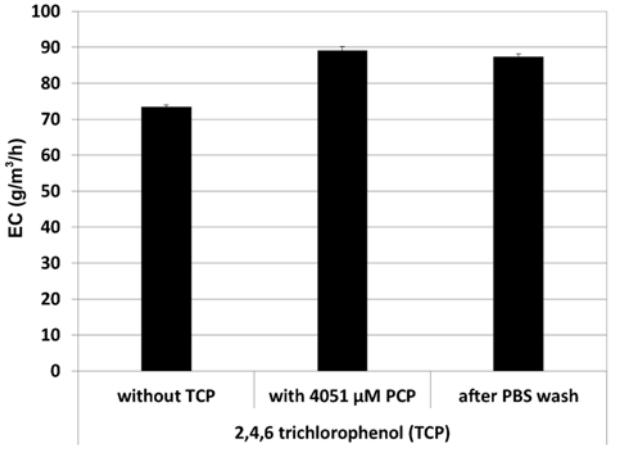

Fig. 6. Effect of 2, 4, 6 trichlorophenol on toluene degradation rate in differential biofilter reactor with soil. Error bars are the standard deviation between the multiple sample injections in GC.

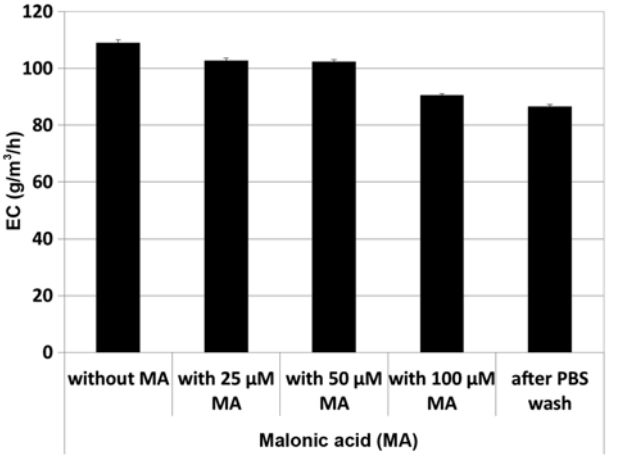

Fig. 7. Effect of malonic acid on toluene degradation rate in differential biofilter reactor with soil. Error bars are the standard deviation between the multiple sample injections in GC.

replaced by 50 μM malonic acid on 40th day which nearly had zero influence on EC and hence on 46th day, 50 μM malonic acid was replaced with 100 μM malonic acid. The EC was decreased by another 11% (Fig. 7). The response of malonic acid was similar to the response of benzoic acid. Hence, the possible explanations for this response are the same as those for benzoic acid. Similar to benzoic acid studies, lower concentrations of malonic acid were not studied in the system, due to the potential malonic acid degradation [19]. Hence malonic acid was considered not suitable as a metabolic uncoupler in enhancing the toluene biodegradation from this system.

3.2.5. Effect of carbonylcyanide p-chloromethoxy phenylhydrazone (CCCP)

Reactor 4 was used for this study. Two cycles of experiments were done with three different concentrations of CCCP. Following a steady state EC (31 g/m^3/h) with soil and PBS after 17 days, a 0.01 μM CCCP solution was added to the system replacing PBS and the EC was reduced by 87%. In order to avoid the complete loss of active toluene degraders, before attaining a steady EC, the 0.01 μM CCCP solution was washed from the system on the 19th day. A series of PBS washes was performed and it was observed that following every PBS wash, the EC increased. However, increase in the EC was not linear with each PBS wash. The maximum EC observed following series of PBS wash was 50 g/m^3/h. After this, further PBS washes did not influence the EC (Fig. 8). A possible speculative reason for this response may be due to the slow diffusion rate of this compound into soil when compared with other metabolic uncouplers which took multiple washes to remove it from soil. However, the diffusion rate of CCCP is unknown. In addition it was observed that reason for the sharp decrease in EC following the addition of CCCP may be that the concentration used in the study was intolerable to the active toluene degraders present in the soil. The experiment was repeated with a fresh soil but at a lower CCCP concentration (0.001 μM). The CCCP addition dropped the EC by 15%. On the 25th day, 0.001 μM CCCP was replaced with a 0.002 μM CCCP solution. Following this change, the EC further dropped by 16% with reference to the earlier one. However, series of PBS washes to remove the 0.002 μM CCCP increased the EC closer to initial EC (Fig. 8). The explanation provided for the similar response in cycle 1 can be again valid for this cycle. But, PBS washes did not increase the EC above the initial EC in cycle two when compared with cycle one. There are two possible speculative explanations for this response of CCCP a) the concentration of CCCP used was inhibiting/killing the toluene degraders and other microbes in soil. When it was removed the slow release of nitrogen had allowed the toluene degraders to bounce back to a higher level than original b) the concentration were inhibitorier (near C_{max}) than killing concentration. Hence CCCP was considered not suitable for similar studies like ours. However, conducting similar experiments in pure culture of toluene degrader (biofilm) in our differential biofilter reactor system will further help to understand the potential uncoupling mechanism of CCCP as the issue of diffusion rate would be nullified in biofilm studies to an extent.

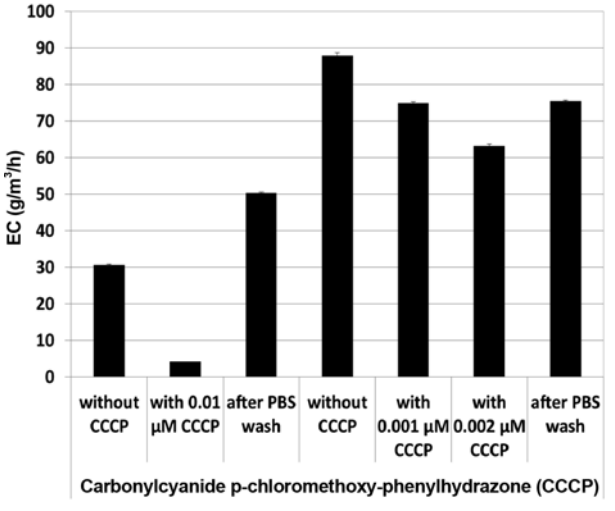

Fig. 8. Effect of carbonylcyanide p-chloromethoxy phenylhydrazone on toluene degradation rate in differential biofilter reactor with soil. Error bars are the standard deviation between the multiple sample injections in GC.

3.2.6. Effect of carbonyl cyanide p-trifluoromethoxy-phenylhydrazone (FCCP)

Reactor 4 was used for this study. Since FCCP belongs to the same family as CCCP, it was decided to test FCCP only at one concentration to understand its effect on the EC. Following the steady state EC of 25 g/m^3/h after 13 days, 0.01 μM FCCP was added to the system by replacing the PBS. The EC increased to 30 g/m^3/h which was 20% higher than the initial EC. Following the removal of FCCP and subsequent PBS washes, the EC dropped to 27 g/m^3/h (Fig. 9). Experiments at higher FCCP concentrations were not performed due to solubility limits. A further lower concentration of FCCP was not tested in the system under the assumption that a lower concentration either would little influence on the EC. When comparing the response of CCCP with FCCP, they behaved differently though both belong to the same family of compounds and have similar pKa values. In particular when compared to CCCP, FCCP behaved as a classic uncoupler in increasing the EC. But to be a 100% classic uncoupler, the response is expected to be reversible with a return to the initial EC upon removal, which was not the case here. Conversely, the diffusion rate

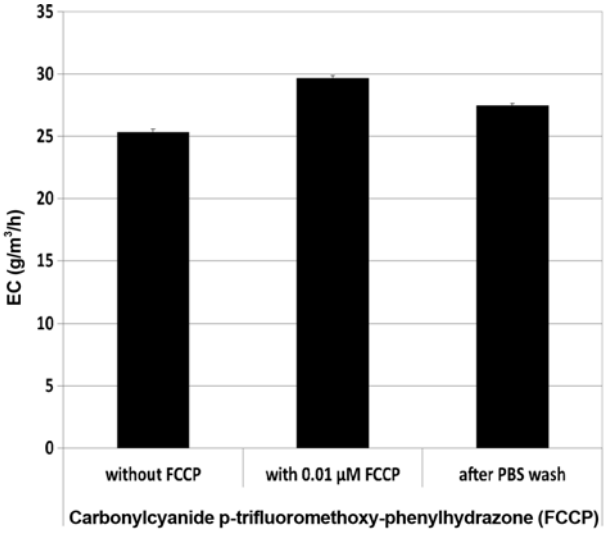

Fig. 9. Effect of carbonyl cyanide p-trifluoromethoxy-phenylhydrazone on toluene degradation rate in differential biofilter reactor with soil. Error bars are the standard deviation between the multiple sample injections in GC.

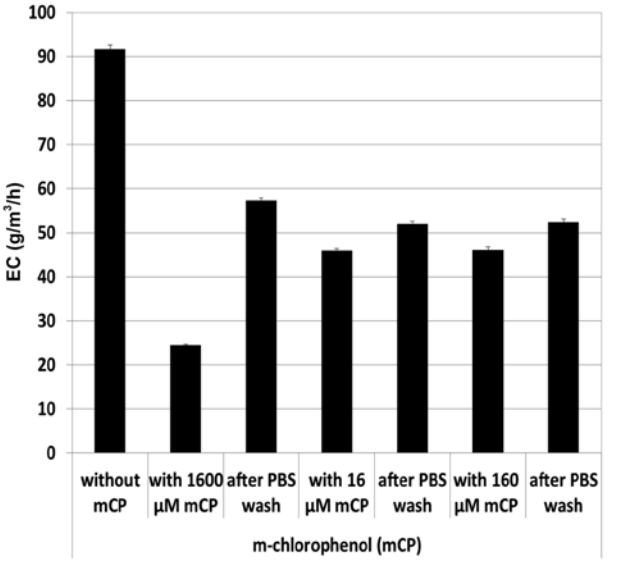

Fig. 10. Effect of m-chlorophenol on toluene degradation rate in differential biofilter reactor with soil. Error bars are the standard deviation between the multiple sample injections in GC.

of FCCP in soil is unknown. Based on the results from CCCP and FCCP studies, the diffusion rate of FCCP may be assumed higher than that of CCCP in soil which can be further correlated to their different response. Though FCCP did not increase the EC significantly in our soil differential biofiltration system, conducting similar experiments in pure culture of toluene degrader (biofilm) in our differential biofilter reactor system will further help to understand the uncoupling mechanism of FCCP clearly.

3.2.7. Effect of m-chlorophenol (mCP)

Reactor 4 was used for this experiment. Initially it was thought that mCP would be similar to PCP and TCP and hence the experiment was started at a higher concentration of 1,600 µM after the initial steady EC value of 24 g/m^3/h on the 9th day. But the addition of mCP dropped the EC by 73%. However, this drop in EC was not similar to CCCP as the decreased EC remained constant in the current case. Hence it was decided to wash with PBS after removing the mCP and to use the same soil to test the lower concentrations of mCP in order to understand its influence on EC. After the removal of mCP and series of PBS washes, the EC recovered to 37% of the initial EC. Following this, experiments were conducted at lower mCP concentrations (16 and 160 µM). None of the concentrations increased the EC value above the initial EC and not even above the earlier EC. Moreover, a series of PBS wash following the removal of 160 µM mCP did not show any significant change in the EC (Fig. 10). It is clear from this experiment that the initial concentration tested was toxic to the active toluene degraders and further reduced concentration tested was slightly inhibitory to the toluene degraders present in the soil. Hence it was concluded that mCP was not a potential metabolic uncoupler for enhancing the toluene degradation.

4. Conclusion

It was observed from the initial screening studies in serum bottle that in a 60 h period, pentachlorophenol, benzoic acid, p-nitrophenol, 2, 4, 6 trichlorophenol and m-chlorophenol increased the toluene degradation rate by 40% compared to the control soil with toluene degraders and 200% compared to the control soil without toluene degraders. The rest of the uncouplers did not work as efficient as those one reported above. Hence the batch mode serum bottle studies helped to select the potential uncouplers in a short time for the further screening studies in continuous mode. From the screening studies conducted in continuous reactor, it was observed that the increase was less than 50%. Moreover, only PCP and TCP increased the EC significantly when compared with FCCP. None of these three metabolic uncouplers behaved reversibly as a classical uncoupler though FCCP showed closer signs of reversibility following PBS washes. In addition the metabolic uncoupler solutions (PCP and TCP) assayed following the experimentation showed decreased concentration when compared with the initial concentration tested. This may be either due to the lower solubility of these chemical which might have caused some residual amount of these metabolic uncouplers to stay in the soil even after multiple PBS washes. Other

metabolic uncouplers tested did not increase the EC and were inhibited the EC. However among these, the response of CCCP was totally different when compared to other 6 uncouplers tested. The EC increased for CCCP tested soil following PBS washes which was higher than the initial EC. Other than the possibilities of lower diffusivity of CCCP (which is not known), the reason for this response is unclear. Overall the response of metabolic uncouplers in growth mode (batch) and maintenance mode (continuous) was clearly distinguished. Since, the major intention of the current research is to increase the maintenance requirement of the toluene degraders in continuous mode and thereby to increase the specific substrate degradation rate, conducting similar studies in pure cultures of toluene degraders in pure culture biofilm reactors will help further to understand the exact biology of the effect of all these metabolic uncouplers.

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