

Effect of critical operational parameters of a biofilm-based bio-filter reactor in enhancing the surface elimination capacity of toluene

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Abstract

The study of biodegradation of Volatile Organic Compounds (VOCs) in a biofilm bio-filter reactor is poorly understood. Toluene is considered as a model pollutant in the current study as it is one of the well-studied VOCs in traditional bio-filters. The extent of toluene in the pollutants is quantified to understand the operation, modeling, and performance of a biofilm bio-filter reactor. Although bio-filtration is simple process, process optimization is most critical in the operation of bio-filters. Henceforth, this study investigates the bio-degradation rate of toluene influenced by critical operational parameters like inlet concentration, pollutant flow rate, precise control of temperature, matric potential, and nutrients in the biofilm bio-filter reactor.

Keywords: Bio-filter; Biofilm; Toluene; Environmental parameters; Surface elimination capacity.

1. Introduction

Biological air pollution control methods have many operational and cost advantages over conventional physico-chemical methods. Biofiltration has been used for waste treatment for almost 100 years, particularly in the treatment of highly concentrated effluents. It is one of the air pollution control technologies (APCT) often used to control odor and volatile organic compounds (VOC's) in outlet gases. Volatile organic compounds (e.g. toluene) with larger air flowrate ($> 1000 \text{ m}^3/\text{h}$) and low concentrations ($< 1000 \text{ ppm}$) can be removed economically using this technology. Polluted air is blown through a porous media in biofiltration, typically a mixture of compost, soil or wood chips that supports a microbe population. Selected microorganisms mostly convert the absorbed biodegradable contaminants into carbon dioxide, salt, and water under optimum conditions. Moreover, in biofiltration the microbial biomass remains static/immobilized on the filter bedding material, while the treated fluid is mobile and passes through the filter. Biofilters are good at handling volatile organic pollutants however its less degradation efficiency has been a problem till date. While biofiltration is a simple process, it depends on many factors that are considered most critical for its operation. They include inlet pollutant/substrate concentration, temperature, pH, moisture content, pressure drop, bed porosity, air flow rate, packing materials, nutrient concentration, acclimation time, oxygen requirement, type of microorganism and residence time. Biofilm based bio-filter reactor systems will greatly benefit in industries in effectively cutting short significantly the size of the traditional biofilters and to effectively treat the gaseous pollutant under a controlled environment. However, critical parameters like inlet concentration, flow rate, temperature, matric potential, and nutrients need to be optimized for achieving good biodegradation rate or surface elimination capacity (SEC). Hence the aim of this work to study the effect of those critical parameters on toluene biodegradation rate in the biofilm biofilters reactor.

1.1 Substrate concentration

The amount of pollutant treated in a bio-filter can be quantified from the inlet concentration of pollutant and its flow rate. Biofiltration is more effective for easily biodegradable pollutant gases (e.g. benzene, toluene etc.) with a dilute (concentrations $< 1000 \text{ ppm}$). On the other hand, biofiltration is less suitable for treating moderately or poorly biodegradable pollutant gases (e.g. dimethyl sulfide, dichloroethane, etc.) at a higher emission concentration [1, 2]. Some researchers hypothesized that the effect of inlet pollutant concentration on the mass transfer rate can be studied

and these reports suggest that the mass transfer rate of the pollutant to the water/biofilm can be improved by increasing the inlet pollutant concentration [3, 4]. However, the metabolic activity of the microbial consortium present in the bio-filter bed may be inhibited by some recalcitrant pollutant gases at high concentrations [5]. Moreover, the air stream containing a high inlet concentration (below the inhibition level) enhances production of biomass along with the addition of nutrients, which significantly prevents the air flow and creates channeling in the bio-filter bed [6, 7]. Information about the influence of pollutant/substrate concentration is required to clearly understand the effect of that pollutant/substrate on the biodegradation rate.

1.2 Temperature

In biofiltration, it is important to maintain an optimum temperature because only over certain temperature ranges the microorganisms present in the biodegradation reaction can show maximum activity. An increase in bio-filter bed temperature generally increases the rate of pollutant degradation until an optimum is reached. Conversely, for most gases, the gas solubility decreases in the aqueous phase, thus reduces the availability of contaminant to the microbes [8]. The biodegradation that takes place in a bio-filter is an exothermic process and so it results in the addition of heat to the bio-filter bed, thus, it also contributes to the overall temperature in the biofiltration process. At the highest cell activity, the temperature of the bio-filter bed increases, which is generally observed in a temperature range of 30-40°C for degraders of toluene [9]. Few researchers suggest that 40°C is the optimum operating temperature for biofilters [10, 11]. However, to attain a maximum rate of degradation, the optimum temperature range will vary based on the type of microorganisms involved in the degradation of a specific pollutant.

1.3 Matric potential

Researchers have identified the importance of moisture in biofilters. Excess moisture in the medium may result in a drop in high pressure, increased limitation of mass transfer, formation of anaerobic zones, and leachate production. Drier bed material resulted in reduced microbial activity, compaction, and channeling, and the formation of hydrophobic material resulted in irreversible drying. Drier bed material resulted in reduced microbial activity, compaction, and channeling, and the formation of hydrophobic material resulted in irreversible drying. To improve metabolic activity and achieve higher removal efficiencies in biofilters, it is therefore considered necessary to maintain moisture content at optimum levels. Optimum values of the moisture content depend on the composition of the media and the physical characteristics of the treated compound. For organic media, for the proper functioning of biofilters, moisture content of 40 to 60 percent (wet weight) was recommended. Research into the causes of changes in moisture content was complemented by observing gradients of water content in biofilters. Very few research works have directly determined how degradation is affected by the moisture content. Microcosm experiments conducted for toluene degradation in a peat medium showed reduced degradation at low water content, although there was sufficient water activity to maintain the metabolism. The low absorption capacity of the material together with low water activity at reduced moisture content was attributed to a decrease in ethanol elimination capacity with a peat medium. Isopentane compost degradation indicated an irreversible decrease in performance below a water content of 0.58 g/g dry weight and a drop-in removal efficiency above optimal values of 0.65 g/g dry weight for moisture content. During the experiment, most of the reported work did not actively control the moisture content. In addition, none of the reported works are linked to a biofilm-based bio-filter reactor [12].

1.4 Nutrients

The bed material in a bio-filter is generally soil, peat, bark, compost, or other materials containing different indigenous microorganisms [5, 13]. These materials not only give physical support to the microorganisms, but also supply some amount of minor and trace nutrients. To maintain microbial activity and consequent pollutant degradation, the biofiltration medium should contain sufficient nutrients. However, the continued supply of nutrients beyond the desired quantity can lead to undesirable problems such as excessive biomass growth with possible obstruction [14].

Microorganisms present in the bio-filter bed utilize the carbon present in the pollutant (e.g. VOCs) as a carbon source for cell material, as carbon is the most important building block in any cell [2]. Biofilters which treat non-carbon containing pollutants (e.g. NH_3 , H_2S etc.,) are mostly autotrophs and hence sometimes they need to be supplemented with additional sources of carbon. Nitrogen is the most essential and important nutrient after carbon for the growth of microbes present in the bio-filter bed. It constitutes about 15% of the dry cell biomass and is a major constituent of proteins and nucleic acids. Most of the microorganisms can use ammonia, and some can also use nitrate as their sole nitrogen source. A significant fraction of the nitrogen used by microbes are recycled after organisms lyse or die [5].

The influence of nitrogen concentration and its chemical nature on bio-filter performance has been frequently reported [15, 16]. Around 59% increase in the removal efficiency is reported in a bio-filter treating hexane after the addition of potassium nitrate to the bio-filter bed [17]. There are also several reports on the use of gaseous form of ammonia as a nitrogen source to the bio-filter bed to increase the biodegradation rate by ten folds [18, 19].

Following nitrogen, phosphorus, sulfur, and potassium are considered critical for many intracellular processes in a microorganism [6, 7, 13]. Addition of phosphorus increased the removal efficiency by 70% in a compost bio-filter [17]. However, there are no reports that sulfur or potassium addition increased the performance of a bio-filter [12]. In addition, it is necessary to maintain a critical ball-park concentration for all these macronutrients in a bio-filter to maintain the normal microbial metabolism [20]. Furthermore, to the macronutrients, cells require micronutrients like magnesium, calcium, vitamins, iron, zinc, copper, and molybdenum in the form of trace elements for maintaining different metabolic pathways. For this reason, micronutrients are frequently added along with macronutrients to bio-filters in minimum quantities [14].

Though both macro- & micronutrients are essential in bio-filter media for increased bio-filter performance, there is no consensus on the optimal concentration required for each of these nutrients. Frequent top up of these nutrients is needed to maintain good bio-filter performance during higher inlet loads or in a long run [17, 21, 22]. Therefore, the frequency, concentration and type of nutrients required for treating different gaseous pollutants/substrates with various bio-filter bed media remains highly empirical.

2. Materials and methods

Biofilm bio-filter reactor with water control system (Figure 1 and Figure 2), offline gas chromatography (Mayura analytical model 1100) and carbon dioxide analyzer (Mayura analytical model 7722) for studying the effect of the substrate, nutrients, temperature and matric potential was designed with reference to the earlier work done [23]. Developed reactor was autoclaved at

121°C with pressure 15 psi for 15 min. Then reactor was loaded with the biofilm under sterile conditions.

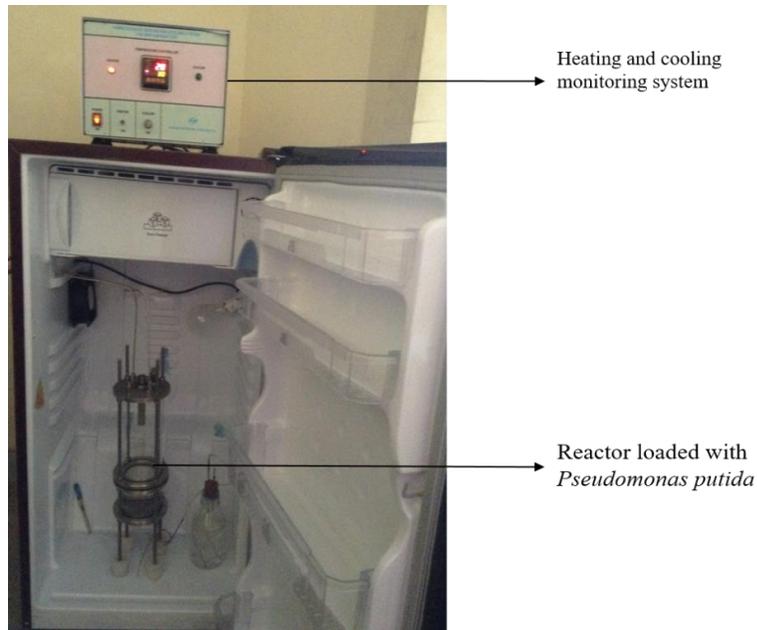


Figure 1. Biofilm bio-filter reactor with offline gas chromatography and carbon dioxide analyzer

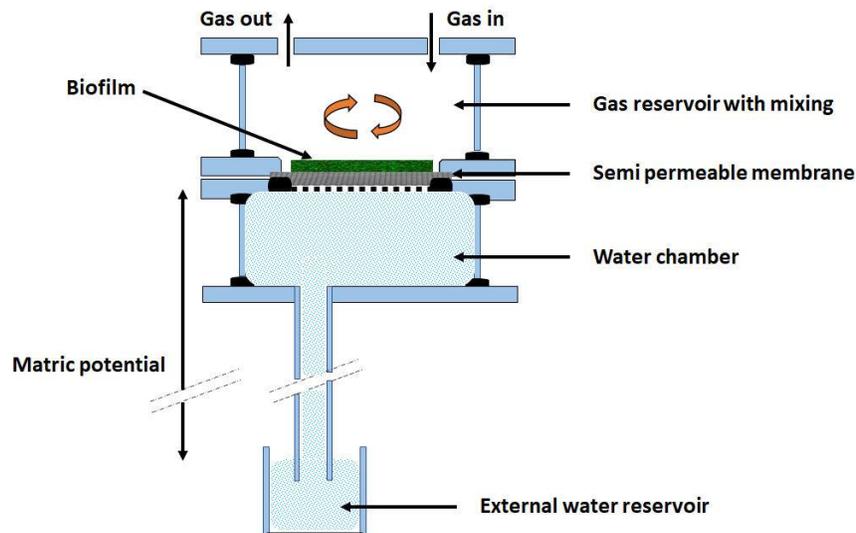


Figure 2. A cut section of the biofilm biofilters reactor with water content control

2.1 Screening of Micro-organism

From the previous work [24], only *Pseudomonas putida* was found to be extensively studied and reported to follow Catabolic pathway of degrading toluene [25]. So that *Pseudomonas putida* MTCC 10617, *Pseudomonas putida* MTCC 1194 and *Pseudomonas putida* MTCC 7426 were

procured from Microbial Type Culture Collection (MTCC), Chandigarh, India. Minimal salt medium (MS medium) [26] was used to prepare culture plates. Sterility was maintained. All the mentioned organisms were individually inoculated in sterile culture plate and incubated at 37 °C in the closed system (3 liter desiccator was used) containing petri-plate with 20 ml vacuum pump oil and 2 µl of toluene (produces 650 ppm of toluene) as carbon source. The plates were monitored daily for its growth and also fresh toluene was added to the vacuum pump oil. *Pseudomonas putida* MTCC 10617 was found to have potential to grow in such concentration of toluene. Hence *Pseudomonas putida* MTCC 10617 was selected as best toluene degrader for making biofilm.

2.2 Loading biofilm in reactor

Pseudomonas putida MTCC 10617 was inoculated in Luria Bertani medium and incubated at 37 °C under toluene environment similar to the screening studies. The initial concentration of *Pseudomonas putida* MTCC 10617 was 1×10^3 Cells/L. Growth rate was monitored every day and at late logarithmic phase, culture was used for the biofilm preparation. Nitrocellulose membrane of about 0.2 µm pore size with surface area of 0.0043 m² was used as a surface for the biofilm. Under sterile condition, 20 ml of pure culture was poured over the membrane in such a way to form uniform biofilm. Then the membrane with biofilm was placed over the sieve plate of the reactor (i.e., between gas and liquid reservoir).

2.3 Generating toluene vapour

Toluene vapour used in this work was generated by simple diffusion system [27], 10 ml diffusion tube with the length 90 mm and 50 mm from the neck were used. Inner diameter of the tube was 3.5 mm. One-liter reagent bottle with diffusion tube inside was placed in water bath with temperature control. By adjusting the temperature of the system and flow rate, length of the diffusion as constant, desired concentration of toluene gas was generated and used for the experimentation.

2.4 Substrate concentration studies

Biofilm bio-filter reactor was operated for 4 months. The concentrations of inlet were changed until a steady SEC was observed at each concentration. Inlet concentration of toluene was varied between 55.20 ± 0.43 ppm and 489.09 ± 2.40 ppm by varying the water bath temperature between 5 °C and 55 °C during this study. To generate lower inlet toluene concentrations, an additional cooler was connected to the water bath for working below the room temperature.

2.5 Temperature studies

The temperature of the insulated box that contains biofilm bio-filter reactor was maintained between 20 °C and 50 °C. To obtain temperatures near ambient and lower, a cooling load through a refrigeration unit attached to the insulated box and a temperature controller turning a 60W light bulb off and on were added. For observation above the ambient temperature, the refrigeration unit was removed. The experiment was carried out by changing the biofilm bio-filter reactor temperature at an interval of 50 °C until a steady SEC was observed.

2.6 Matric potential studies

The effectiveness of the biodegradation process is influenced by the optimal water content. The stagnant zones are formed when very high moisture content is present that limits diffusion of nutrients transportation and possible anaerobic conditions. The microbial activity is limited by lesser water content [28]. This parameter can be studied by operating the biofilm bio-filter reactor at 40° C at water tensions of 10 cm_{H₂O} and 20 cm_{H₂O}.

2.7 Nutrient addition

Different nutrient solutions (Table 1) were added to the biofilm bio-filter reactor bed by using the external lower water reservoir by connecting it hydraulically with the internal upper reservoir (the one below the membrane). The experiment was performed after autoclaving all the nutrient solutions¹ at 121 °C for 45 minutes. Phosphate buffered saline (PBS) washes were performed before swapping the nutrient solutions in the reactor.

Table 1. Addition of different nutrients to the biofilm bio-filter reactor for determining the impact on surface elimination capacity

Nutrient	Concentration (g/L)
Nitrogen Source	
NaNO ₃	4.00
Phosphate Source	
KH ₂ PO ₄	0.24
NaH ₂ PO ₄ .H ₂ O	1.44
Sulphate, Magnesium, Ferrous Sources	
MgSO ₄ .7H ₂ O	0.2
FeSO ₄ .7H ₂ O	0.0008
Calcium Source	
CaCl ₂ .2H ₂ O	1.42
Tap water	NA

3. Results and discussions

3.1 Substrate concentration effect

The effect of concentration of inlet toluene on SEC was investigated by manipulating concentrations of inlet toluene to change the load. The experiments were conducted on the

¹Except tap water and calcium chloride all other nutrient solutions were prepared in 1X PBS (buffered at pH: 7.0) for eliminating the effect of pH on degradation of microbes.

assumption that, unless nutrients were added, biomass would not change in the biofilm. The biofilm bio-filter reactor was operated for 150 days before these experiments were started, and any excess nutrients to stimulate growth were assumed to be exhausted.

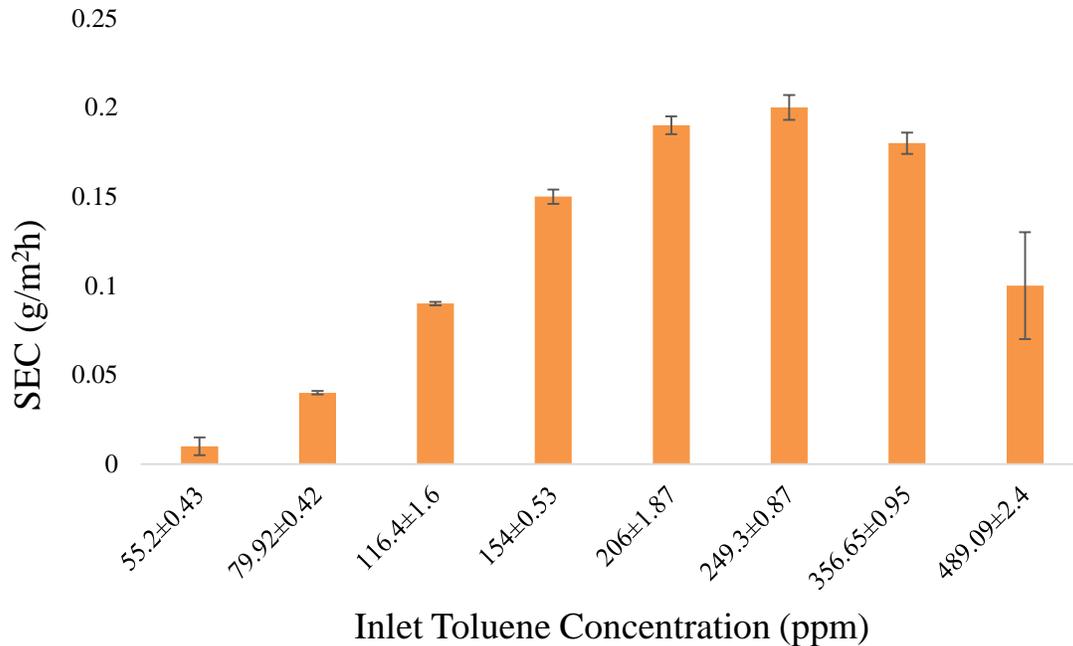


Figure 3. The relationship between the inlet toluene concentrations on the SEC. Error bars are the standard deviations

The biofilm bio-filter reactor was operated at an inlet toluene concentration ranging between 55.2 ± 0.43 ppm and 489.09 ± 2.4 ppm (Figure 3). It was observed that a lower steady SEC of 0.01 $\text{g/m}^2\text{h}$ was attained at 55.2 ± 0.43 ppm and a higher steady SEC of 0.2 $\text{g/m}^2\text{h}$ was attained at 249.3 ± 0.87 ppm. Further increase the inlet toluene concentration significantly reduced the SEC which confirmed that the biofilm cannot handle any increase in the inlet toluene concentration beyond the threshold value of 249.3 ± 0.87 ppm. Based on few earlier reports, oxygen limitation in a bio-filter is unlikely to influence the SEC at these loads [29, 30] and hence the current response can be attributed to substrate inhibition.

3.2 Temperature effect

The operating temperature of the biofilm bio-filter reactor was increased stepwise from 20 °C to 45 °C during the experiment. Increasing the temperature of the reactor increased the SEC to a maximum of 0.2 $\text{g/m}^2\text{h}$ at 40 °C (Figure 4). However, the SEC started to drop steeply above 40 °C. The average inlet toluene concentration at this point was observed as 249 ± 3 ppm. In addition, it was also observed that the increase in SEC was gradual between 20 °C and 40 °C but after that SEC started to drop steeply. However, a similar study reported that maximum specific toluene degradation rate was observed at 30 °C in a bio-filter [31]. Hence from the current study it can be concluded that a highest intensity of the metabolic microbial activity in biofilm was seen at 40 °C.

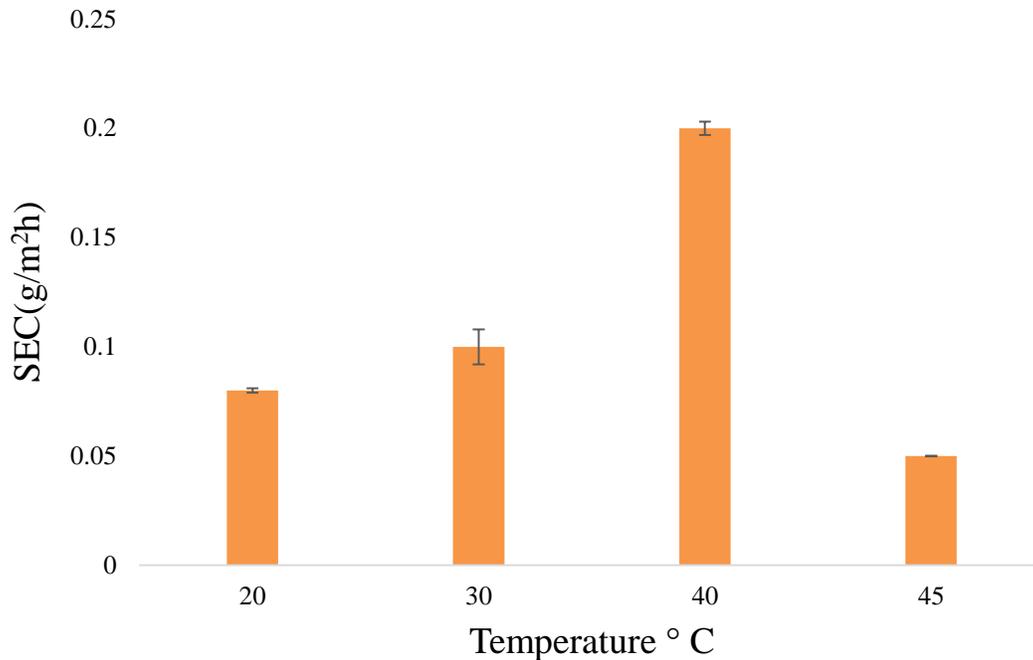


Figure 4. Effect of temperature on surface elimination capacity. Error bars are the standard deviations

3.3 Matric potential effect

Matric potential in the biofilm bio-filter reactor was increased stepwise from 10 cm_{H2O} to 25 cm_{H2O} during the experiment. Increasing the matric potential of the reactor increased the SEC to a maximum of 0.2 g/m²h at 20 cm_{H2O} (Figure 5). However, it started to decrease significantly beyond 20 cm_{H2O}. In addition, significant difference in carbon recovery as CO₂ was found with 55% at 10 cm_{H2O} as opposed to 30 % at 20 cm_{H2O}. Holden *et al* [32] has previously attributed reduction in growth rates during soil drying to matric potential in the presence of a VOC carbon source. However, a higher fraction of 50% carbon was recovered in the solid and liquid phase at 20 cm_{H2O} run and 32% was recovered for the 10 cm_{H2O}. Most likely, this carbon fraction is a combination of extracellular polysaccharides (EPS), microbial soluble products, and dissolved CO₂. Water stress response also results in polysaccharides being produced. These types of water stress response, such as polysaccharides and solutes production, involve cellular energy and should decrease the cell synthesis balance. Since this study was a non-growth system microbe, due to the production of storage polymers and other soluble microbial products (SMP) could be a plausible explanation for carbon recovery at higher voltage in the solid and liquid phase.

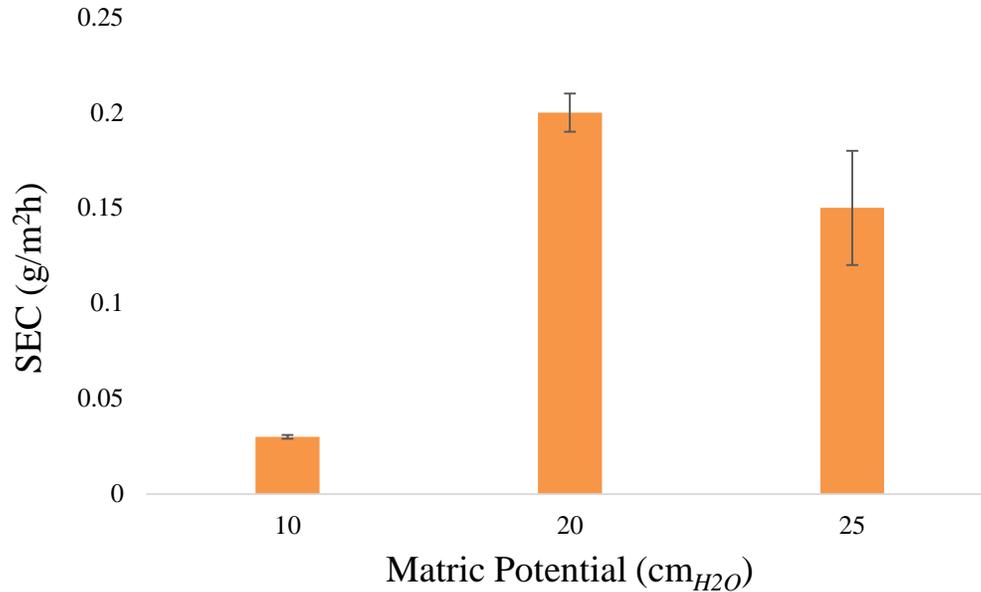


Figure 5. Effect of matric potential on surface elimination capacity. Error bars are the standard deviations

3.4 Nutrient effect

Before starting the nutrient studies in the biofilm bio-filter reactor, the reactor was operated for 14 days as an acclimation period for toluene degraders present in the biofilm. Moreover, the reactor was started with tap water initially. A steady SEC was observed after the 14th day with tap water (Figure 6). In the current study, tap water was considered as a control and the corresponding SEC was considered as control SEC (0.02 g/m²h). To avoid possible contamination in the liquid phase, all the nutrient solutions (including tap water) used in this experiment were autoclaved before introducing into the reactor. In addition, other than the tap water and calcium chloride, all other nutrients were buffered, and the pH was adjusted between 6.5 and 7.5 prior to introducing them into the reactor.

Following the steady state SEC (0.02 g/m²h) with tap water, at day 15, 0.01 M calcium chloride [33] was added after removing the tap water which slightly increased the SEC. The steady state SEC observed following the addition of calcium chloride was 0.025 g/m²h. Since this marginal increase in EC was not considered significant, the toluene degrader present in the biofilm was not calcium limited. After achieving steady state SEC at day 31, phosphate buffered saline (PBS) was added by removing the calcium chloride from the reactor. Slight decrease in the SEC was observed after 14 days (day 45) of PBS addition. The steady state SEC after PBS addition was observed as 0.01 g/m²h. Because this marginal decline in SEC was not considered significant, it was considered that PBS had almost nil influence on SEC, like calcium chloride. Following this experiment, the decision was made to prepare all experimental solutions in PBS and, before loading into the reactor, the pH of all test solutions was adjusted to 7.0. In addition, when a new test solution was loaded and removed from the reactor, PBS washes were performed. This was done to remove the microbial degradation pH effect. After the day 70, PBS was replaced by 0.05 M sodium nitrate which increased the SEC 40-fold after 15 days. The steady state SEC was observed following the

addition of sodium nitrate was $0.4 \text{ g/m}^2\text{h}$. This response proved that nitrogen was the substrate limiting the growth of toluene degrader present in the biofilm bio-filter reactor. Nitrogen limitation has been observed in other research related to the treatment of different volatile organic compounds (VOCs) in different biofilters [12, 17, 34-36]. The sodium nitrate solution was replaced with PBS water after a new steady state SEC was achieved to get a new SEC control value on day 69. There was no significant change in the SEC and it clearly showed that the increase in SEC during the addition of sodium nitrate was only due to the growth of biomass. PBS was replaced by magnesium sulphate and ferrous sulphate solution on the 74th day and no further changes were observed in SEC. This has shown that the toluene degrader is not limited to Mg/Fe or sulphate. It was also clear from these studies that, in addition to controlling the water content of the bio-filter bed, the current experimental setup provided an easy and controlled environment for nutrient addition and removal. Since all nutrient solutions were autoclaved before use and both internal and external water reservoirs were sealed, the nutrient solutions potential for microbial growth was minimized. Therefore, studying the nutrient effect in the current experimental setup was relatively easy.

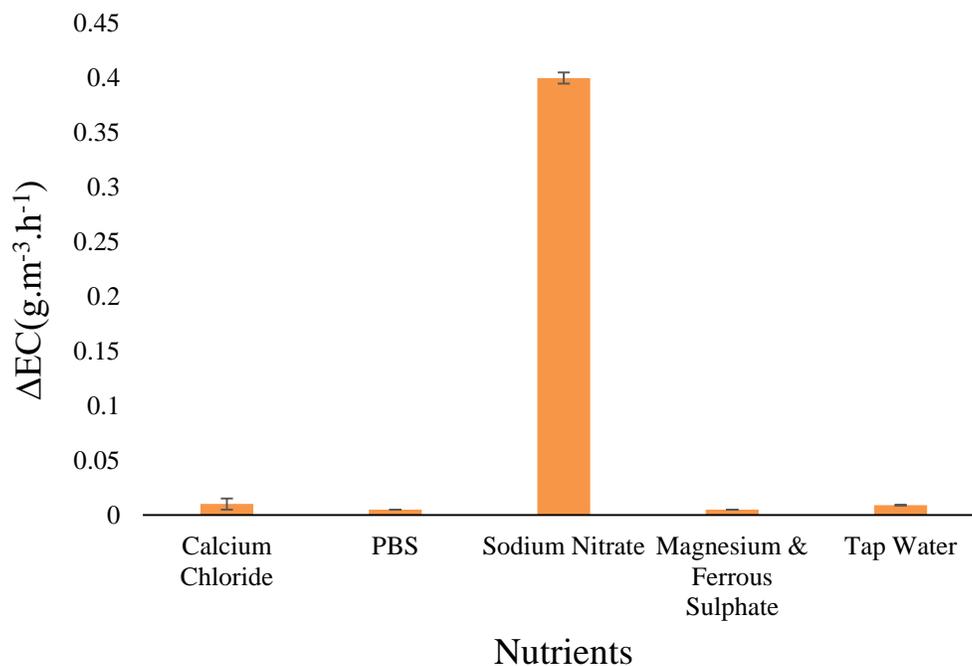


Figure 6. Overall results of nutrient effect on the SEC in the biofilm bio-filter reactor. Error bars are the standard deviations

4. Conclusions

The biofilm bio-filter reactor used in the study showed a high degree of flexibility in manipulating critical parameters, such as substrate concentration, temperature, matric potential, and nutrients at a fixed inlet toluene gas flow rate. From the substrate concentration studies, it was demonstrated that substrate inhibition was dominant above 249.3 ± 0.87 ppm. Temperature studies showed that the SEC of the biofilm bio-filter reactor increased with increasing temperature, from $0.08 \text{ g/m}^2\text{h}$ to $0.2 \text{ g/m}^2\text{h}$ for temperatures of 20 to 40 °C, respectively. This increase in SEC was due to an

increase in the activity of the toluene degrader present in the bio-filter bed. Experiments carried out at three different matric potentials proved that at a threshold matric potential of 20 cm_{H2O}, maximum SEC of 0.2 g/m²h was observed. Studies conducted using different nutrients clearly showed that nitrogen was limited for the toluene degrader present in the biofilm. This was evident from SEC's 40-fold increase under the influence of the source of nitrogen, but the other nutrients showed no significant increase in SEC.

CRedit authorship statement

Vishal Bellie Subramani: Conceptualization, Methodology, Data curation, Writing -original draft & editing. **Suganya Baskaran:** Investigation, Visualization, Writing & Editing. **Palani Ramasamy:** Validation, Formal analysis. **Swaminathan Detchanamurthy:** Funding acquisition, Project administration. **Parthiban Rangasamy:** Supervision.

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Declaration of competing interest

The authors declare that they have no known financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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