Biodegradation of mono-chlorobenzene by using a trickle bed air biofilter (TBAB)

Anil K. Mathur*, C. B. Majumder, Dhananjay Singh and Shashi Bala

1Biotechnology (Applied Mechanics Department), Motilal Nehru National Institute of Technology, Allahabad - 211 004, India
2Chemical Engineering Department, Indian Institute of Technology, Roorkee-247667, India
3Chemical Engineering Department, Institute of Engineering and Technology, Lucknow - 226 021, India

(Received: March 05, 2009; Revised received: July 31, 2009; Accepted: August 26, 2009)

Abstract: In the present study, performance of the trickle bed air biofilter (TBAB) for treating mono-chlorobenzene (MCB) was evaluated for various influent volatile organic compound (VOC) loadings using coal and mixed consortium of activated sludge as the packing material. Microbial acclimation to MCB was achieved by exposing the system continuously for 31 d to an average inlet MCB concentration of 0.688 g m\(^{-3}\) at an empty bed residence time (EBRT) of 188 s. The TBAB achieved maximum removal efficiency of 87% at an EBRT of 188 s for an inlet concentration of 0.681 g m\(^{-3}\), which is quite significant than the values reported in the literature. Elimination capacities of MCB increased with an increase of the influent VOC loading, but an opposite trend was observed for the removal efficiency. The maximum elimination capacity of the biofilter was 110.75 g m\(^{-3}\) hr\(^{-1}\) at an inlet MCB concentration of 1.47 g m\(^{-3}\). The effect of starvation on the TBAB was also studied. After starvation, the TBAB lost its ability to degrade MCB initially. However, the biofilter recovered very quickly. Evaluation of the concentration profile along the bed height indicated that the bottom section of TBAB has the best performance for all concentrations. By using Wani’s method of macrokinetic determination based on simple Monod kinetics, the maximum removal rate of MCB, \(r_{\text{max}}\), and saturation constant \(K_s\) was to be found as 1.304 g m\(^{-3}\) hr\(^{-1}\) and 113.446 g m\(^{-3}\), respectively.

Key words: Trickled bed air biofilter (TBAB), Mono-chlorobenzene (MCB), Empty bed residence time (EBRT), Monod kinetics

PDF of full length paper is available online

Introduction

Enormous quantities of air pollutants are being released into the environment by various industries. Volatile organic compounds (VOCs) belong to a special category of air pollutants which are hazardous and carcinogenic, and take part in photochemical reactions and smog formation. The presence of VOCs in the atmosphere creates a number of problems for human health as well as environmental quality. With increasing public concern about deteriorating environmental air quality, stringent regulations are being enforced to control air pollutants. Chlorinated monoaromatic compounds have been used in large quantities as solvents, lubricants, insulators, insecticides, herbicides and plasticizers (Gambilish et al., 1996). The Clean Air Act Amendments of 1990 list chlorobenzene as a hazardous air pollutant. It is used in the production of nitrochlorobenzene, adhesives, paints, paint removers, polishes, dyes, drugs, phenol, pesticides (like DDT) and inkline. Monochlorobenzene (MCB) is a volatile (boiling point = 131°C) and flammable liquid. The escape of its vapours into the ambient air will create to an adverse impact on air quality affecting public health and welfare.

Biofiltration is used as a versatile treatment technology for waste and malodorous gases and VOCs. Its low cost, operational simplicity and lack of secondary pollutant generation makes it a very attractive and popular treatment method. In biofiltration, contaminated gases are passed through beds, packed with solid media that support a biologically active layer. As gases flow through the support medium and pass the biofilm, contaminants transfer from the gas phase into the aqueous and solid phases (e.g., support medium and bio-film) where micro-organisms can biodegrade the contaminants into innocuous products, such as carbon dioxide, water and cell mass (Moe and Li, 2005). Although, many researchers have reviewed the principles of biological waste air treatment and the advantages over chemical and physical techniques, research is still going on to the biological treatment of waste air for the use of effective new packing media, designs, microbial structure analysis and modelling of VOC removal. Furthermore, attempts to genetically modified bacteria to increase the degradation rate of a single pure organism are being made (Iranpour et al., 2005, Mathur and Majumder, 2008). A trickle-bed air biofilter (TBAB) is very popular type of biofilter that employs synthetic inorganic media for the growth of microbial mass and the liquid nutrients and buffers are introduced from the top through a nozzle for its uniform distribution across the bed cross section. Through the control of pressure drop across the bed, pH, and the nutrient feed, TBAB facilitates consistent operation in comparison to natural media biofilters. They also do not suffer from the process of aging, as do natural media (Sorial et al., 1997).

The literature survey reveals that only a few researchers have attempted the treatment of MCB vapour bearing waste air streams in the biofilters and biotrickling filters (Mpanias and Baltzis, 1998; Seigner et al., 2002; Oh and Bartha, 1994; Delhomenie and Heitz, 2003). Studies on the biofiltration of MCB in a coal-based biotrickling filter are relatively scarce in the literature. Mpanias and
Baltzis (1998) investigated the treatment of MCB in a biotrickling filter packed with 3/4" intolax ceramic saddles and reported a MCB removal efficiency of 90% from its initial concentration 2 g m$^{-3}$ in air. Seignez et al., 2002 obtained a maximum MCB removal efficiency of 64% and the elimination capacity (EC) of 0.71 kg m$^{-3}$ day$^{-1}$, at mass loading rate of 1.1 kg m$^{-3}$ day$^{-1}$ in a biotrickling filter packed with Sulzer Mellapak. Oh and Bartha, 1994 studied the degradation of MCB and o-Dichlorobenzene in a biotrickling filter packed with perlite. The maximum removal efficiency of MCB and o-Dichlorobenzene was achieved as 65 and 85%, respectively. Delhomenie and Heitz (2003) reported more than 90% removal of MCB with the initial MCB concentration up to 1.2 g m$^{-3}$. The maximum rate was varied from 0.5 to 3.0 m$^{3}$ hr$^{-1}$ with a constant inlet concentration of 1.2 g m$^{-3}$. A maximum EC 70 g m$^{-3}$ hr$^{-1}$ was found for gas contact time greater than 60 s. Varying flow rates were used to evaluate the kinetics of treatment. A first order kinetics was found to fit the experimental data. Biofiltration of isopropanol and acetone were studied in a peat packed biotrickling filter. More than 90% removal efficiencies were achieved with influent carbon loadings of isopropyl alcohol and acetone below 80 and 53 g m$^{-3}$ hr$^{-1}$, respectively (Chungsingy and Kwotsair, 2003).

The purpose of this research is to evaluate the performance of a TBAB with a mixed microbial culture treating a synthetic MCB vapour stream under various operating conditions. The removal efficiency was monitored by varying the process parameters and operating conditions. In addition, the performance of TBAB was also tested.

### Materials and Methods

**Experimental setup:** The trickle bed air biofilter (TBAB) was used for MCB removal. It was made of a transparent Perspex pipe of 50 mm diameter and had a length of 1 m. A 100 mm headspace was provided for the separation of the treated gas from the liquid and for housing the nutrient spray nozzle and 100 mm bottom space was designed for the MCB waste gas inlet and leachate. The coal was used as a packing material. Coal was crushed and sieved with size ranges of 1-1.5 cm. The coal particles had a specific gravity of 2.514 and the BET surface area of 4.898 m$^{2}$ g$^{-1}$. The sieved particles were washed with three times successfully with Millipore water and dried in an oven at 105°C for 1 d. These particles were sterilized in a steam autoclave at 15 psi for 20 min. The particles were filled in the Perspex pipe provided with an acrylic plastic mesh supported on acrylic plate distributed at the bottom of the filter. The filter was filled up to 1.57 x 10$^{-3}$ m$^{3}$ volume with the sterilized coal particles and was inoculated with MCB acclimated mixed microorganisms. All experiments were conducted in a temperature controlled chamber at 30 ± 2°C.

The air stream from the oil-free and particles-free compressor, at a pressure of 7 kg m$^{-2}$ was passed. Split with one stream passing through the humidifier and other through the MCB (99% pure) bottle. The two out going air stream were mixed in a glass mixer and then mixed with the major air streams and passed through the bed. The air flow rates were measured by a rotameter (JTM, Japsin Industrial Instrumentation, India) for high flow rate (0.5-5 l min$^{-1}$) and by a soap bobble flow meter (Netel India Limited) for low flow rate (1-50 ml min$^{-1}$). Air having varying MCB concentration and flow rates was passed through the TBAB. Samples were collected at a regular time intervals from the inlet, outlet and the sampling ports using an airtight syringe and analyzed for residual MCB concentration.

**Microbial consortium:** Activated sludge was from the secondary clarifier at the Kankhal Municipal Treatment Plant at Hardwar, India, was used to inoculate the TBAB. The activated sludge was allowed to settle for 5 hr to obtain the concentrated sludge. The concentrated sludge had suspended solids (SS) concentration of 3000 mg l$^{-1}$ and volatile suspended solids (VSS) concentration of 2100 mg l$^{-1}$. 100 ml concentrated activated sludge was used for the preparation of seed culture in a 250 ml flask. 2% glucose solution was used as the carbon source for two days. After that the 5 ml of activated sludge was contacted with 95 ml nutrient solution containing of 5 μl MCB. All solutions were shaken at 125 rpm and 30°C in a temperature controlled shaker. The MCB adopted culture was transfer of the culture at every 48 hr interval for 6 d. After that 100 ml of this culture was transferred to a 1 l nutrient solution stirred and introduced from the top to the TBAB and recirculated for 12 hr. After that the TBAB was fed with the nutrient solution continuously at a rate of 3-5 ml min$^{-1}$. The pH of the nutrient solution was maintained at 7.0 ± 2 by adding NaOH or HCl. MCB vapour laden air was supplied from the bottom of the reactor.

**Analysis of monochlorobenzene (MCB):** MCB concentration in air was analyzed by using a HP 5895A gas chromatograph equipped with a capillary column type HP 1 (30 m), and with a flame ionization detector (FID). The injector, oven and detector temperatures were maintained at 190, 190 and 200°C, respectively. Hydrogen was used as a fuel and nitrogen was used as the carrier gas at a flow rate of 20 ml min$^{-1}$. Air samples were drawn from the various sampling ports by using a gas tight syringe and analyzed. Suspended solids (SS) and volatile suspended solids (VSS) of activated sludge were determined according to the Standard Methods (APHA, 2005). The pH values of nutrient solution and leachate were measured by a digital pH meter (NAINA NIG-333, India). Scanning electron microscope (SEM) micrographs of coal particle, at the beginning of the experiments and after 110 d operation were taken by using a SEM (Model LE0435VP, LEO Electron Microscopy Ltd, England).

**Measurement of concentration of microorganism:** The plate count technique was used for the measurement of microbial concentration. When the steady state removal of MCB was established, one gram each of solid sample was taken from TBAB at two different locations in the bottom section. Each sample was mixed with 10 ml of Millipore water and then shaken in a vortex shaker for 10 min and then converted to desired concentration through serial dilution technique. Diluted sample was then allowed to stand for 30 min. One ml of this sample was serially diluted up to 10$^{-6}$ in sterile buffer (phosphate buffer, pH 7.0). Serially diluted sample was then spread ascetically on the solid nutrient agar plate and the plates were incubated at 30°C. After 2 d of incubation, the plates were taken out and the colonies were counted (Moe and Qi, 2005; Mathur and Majumder, 2008).
Biodegradation of mono-chlorobenzene in biofilter

Kinetic analysis: In the gas-phase biotrickling filters, the degradation rate of VOCs within the biofilm was investigated. For this reason, the mathematical model expressed by equation (1) was used. Details of method for determination of kinetic constants are presented elsewhere (Kraila et al., 2004).

\[
\frac{V/Q}{C_{gi} - C_{go}} = \frac{K_s}{r_{max}} \frac{1}{C_{ln}} + \frac{1}{r_{max}}
\]  

(1)

where \(C_{gi}\) and \(C_{go}\) is the inlet and outlet MCB concentration, \(C_{ln}\) is the log mean concentration \([\frac{(C_{gi} - C_{go})}{\ln(C_{gi}C_{go})}]\), \(V\) is the biofilter volume \((m^3)\), and \(Q\) is the volumetric flow rate \((m^3/s)\). \(r_{max}\) is the maximum degradation rate per unit filter volume \((g m^{-3} h^{-1})\) and \(K_s\) is the saturation (Michaelis-Menten) constant \((g m^{-3})\) in the gas phase.

Results and Discussion

Performance of TBAB: The inoculated TBAB was fed with MCB laden air at a flow rate of 0.5 l min\(^{-1}\) corresponding to an EBRT of 188 s.
with an inlet MCB concentration of 0.688 g m$^{-3}$. Gradual increase in removal efficiency with the passage of time was observed (Fig. 1), with the removal efficiency reaching about 87% after one month. Inoculation of the biofilter media with adapted microbial aggregates greatly reduces the acclimation time of the biofilter (Shareefdeen and Baltzicm, 1994; Acuna et al., 1999; Jorio et al., 2003) to as low as 12 days (Arnold et al., 1997). Steady-state conditions were observed after day 28 of the operation. The results are consistent with the reported acclimation periods from several weeks to several months (Juneson et al., 2001).

Performance of TBAB was investigated with respect to the loading rate (LR) of MCB in g m$^{-3}$hr$^{-1}$. To do that, loading rate was expressed in terms of the combination of two parameters: MCB concentration (g m$^{-3}$) and flow rate (l min$^{-1}$). These two parameters were actually varied in the reactor to study the performance of the reactor to maintain the predefined loading rate.

Reactor had been operated in the five phases (Fig. 1) over a total time of 110 days. The flow rate and MCB concentration were varied to maintain and regulate the loading of the reactor in different phases. First phase lasted from 1 to 31 day. Flow rate of the gas mixture and average MCB concentration were maintained at 0.51 min$^{-1}$ and at 0.688 g m$^{-3}$ respectively, with a loading rate of 13.14 g m$^{-3}$ hr$^{-1}$. Corresponding EBRT was 188 s. At the end of the first phase, 87% removal of MCB was achieved at the steady state condition. In phase II, loading rate has been increased from 13.14 g to 46.26 g m$^{-3}$ hr$^{-1}$ with the flow rate and average MCB concentration being 0.75 l min$^{-1}$ and at 1.616 g m$^{-3}$ respectively. The sudden increase in the loading rate affected the reactor efficiency adversely with the MCB removal decreased drastically to 44% from the initial value of 87%. However, the performance of the reactor recovered gradually in 5 days to 78% at steady state. Corresponding EBRT was 125 s in second phase. Phase II lasted for almost 17 days. In phase III, the loading rate was further increased from 46.26 g to 128.19 g m$^{-3}$ hr$^{-1}$ with the corresponding flow rate and MCB concentration being 1 l min$^{-1}$ and 3.356 g m$^{-3}$ respectively. The step rise in loading rate regulated in the sudden decrease in the removal efficiency from 78 to 41%. However, the reactor recovered its performance with the MCB removal efficiency increasing to 69% in 5 d. In the latter part of phase III (after 30 days), the loading rate was further increased to 287.86 g m$^{3}$ hr$^{-1}$. This again resulted in the step fall of the removal efficiency from 69 to 37%. This decline in the efficiency continued for the next four days of this phase. Since the reactor could not regain its efficiency of 69% of the first part of phase III, loading rate was decreased by 2 times from 287.86 to 133.89 g m$^{3}$ hr$^{-1}$ on day 82, starting the phase IV. It is quite interesting that the 78% removal efficiency was achieved within 5 days again which was the recovery of the 50% fall in the removal efficiency. Moreover it has been observed that it took 5 days time as the recovery time for any shock loading. Another interesting trend has been observed that for any shock in the loading rate there is almost 40-50% fall in the removal efficiencies. This has been reconfirmed when starvation period was applied to the reactor. During the starvation period only nutrient was supplied from day 91 to 97. Nearer to same loading rate as in the phase IV i.e. 126.68 g m$^{3}$ hr$^{-1}$ was maintained in phase V after the starvation period. Corresponding flow rate and MCB concentration were again 2.5 l min$^{-1}$ and 1.326 g m$^{-3}$ respectively. Percent removal of MCB is same (78%) here also. Almost 3 days time was required after starvation period to regain its activity and a total 5 days were required to get back the maximum activity. Phase V lasted from 97 to 110 d, for 13 d. Moreover, the reacclimatized profile of MCB removal after starvation period was faster than that required for the initial acclimation period. Fig. 2 shows the effects of change in the inlet MCB loading rate in the various phases on the maximum removal efficiencies throughout the experimental period. Maximum removal efficiency has been decreased with the increases in MCB load, where as elimination capacity has been increased with the increase in the load.

Fig. 3 shows a variation in the inlet, outlet concentration and EC of MCB. During operation with inlet MCB concentration of 1.47 g m$^{-3}$ with an EBRT of 37 s, the maximum EC was 110.75 g m$^{3}$ hr$^{-1}$ occurred in the phase IV. Significant variation of the EC in various phases was observed due to change in influent concentration and removal rate. During phase III, the TBAB was subjected to low flow rate and relatively high concentration as compare to phase IV.

Removal of MCB: For determining the local MCB concentration the sampling ports were provided at the various locations along the bed height. The entire bed has equally divided into four sections of 20 cm each. The results presented in Fig. 4 indicate that the total removal of MCB is more efficient in the lower part more than 42% of TBAB than in the upper part of the filter. This was due to the fact that more carbon sources, moisture contents and nutrients were present in the bottom section of TBAB, which caused a higher metabolic reaction and thus led to faster biodegradable rate.

Kinetics analysis: The kinetic parameters have to be calculated in order to understand transport phenomena and kinetic behavior of biotrickling filters. The kinetics of the system can be expressed by a Michaelis-Menten relationship by assuming that oxygen limitation was not present in the system and the conversion takes place in the reaction-controlled regime (i.e. the biofilm was fully active). At the steady state, the growth rate of microorganisms was balanced by its own decay rate, resulting in the biological equilibrium of the system. Hence, kinetic constants remained constant over the period of time considered. The kinetic constants were determined by using Eq. (1) with correlation coefficient ($R^2$) of MCB was 0.9994. From Fig. 5, the $r_{max}$ and $K_m$ value for MCB was 1.304 g m$^{-3}$ hr$^{-1}$ and 113.446 g m$^{-3}$, respectively. Kralias et al. 2002, 2004 also reported, the $r_{max}$ values for methanol and isopropanol were 0.822, and 0.12 g m$^{-3}$ hr$^{-1}$ and $K_m$ values were calculated as 134.9 and 2.72 g m$^{-3}$, respectively.

Microscopic observations: The system achieved steady state removal efficiencies for various experimental phases of operation in Fig. 6. The microbial concentration in initial activated sludge was $2.32 \times 10^{6}$ CFU gr$^{-1}$ and increases 11 fold ($2.57 \times 10^{7}$ CFU g$^{-1}$) after 31 days operation. The microbial concentration increases significantly
**Fig. 3:** Elimination capacity of biofiltration of monochlorobenzene at different inlet concentration

**Fig. 4:** Concentration and removal efficiency profile of MCB along the TBAB column

**Fig. 5:** Kinetic analysis from steady state data on MCB degradation

**Fig. 6:** Total microbial count during various phase of TBAB
from phase I to III. The maximum microbial concentration was achieved as $5.45 \times 10^8$ CFU g$^{-1}$ in the part I of phase III. The microbial concentration decreased from $5.45 \times 10^8$ to $2.12 \times 10^7$ CFU g$^{-1}$ when inlet concentration suddenly increased in the part II of phase III. Due to sudden changed in inlet concentration and the loading rate, the microbial concentration was decreased significantly. However, the microbial concentration recovered quickly to higher levels as soon as the inlet concentration decreased in the phase IV. Again the microbial concentration decreased, when only nutrient was supplied in the starvation period since energy source is very important for survive microorganisms. The microbial concentration again recovered quickly to higher levels when conditions were maintained in the phase V.

The Scanning Electron Micrograph (SEM) can provide crucial quantitative and qualitative information about the microbial community on the biofilter media. The biomass of individual particle can be mapped. The SEM can also determine the characteristic and activity of the filter material that are important to system’s success. If the filter bed is showing increases in pressure drop or poor performance because of channelling, SEM may show whether clogging is arising from accumulation of biomass (Divenny et al., 1999). The SEM of microbial growth on various types of media before and after experiment has already been shown by some researchers (Rene et al., 2005; Namkoong et al., 2004). Fig. 7 present microbial growth on the surface of the coal before and after experiment. Compared to the initial coal media, a biofilm on the surface of the coal was observed clearly after 110 days of operation. An even growth of microbial community on the surface of the pore of the coal is clearly visible. Inspection of higher magnification SEM image (on the order of $\times 1000$), however, revealed that large number of rod shaped bacteria were present in the biofilm growing attached on the surface of coal. Initially, the degree of acclimatized depends upon the adaptive capacity of the microorganism in the coal, substrate concentration and its availability and other necessary environmental condition like pH, temperature. Acuna et al., 1999 reported that, after 88 days of biofiltration, diverse microbial morphologies, such as bacterial colonies, single cell and budding yeasts, mycelial structures, and also some non-colonized regions on the surface of a peat biofilter were observed under SEM. Even though biofilms seem to consist of a homogeneous layer, there is a considerable non-uniformity within biofilms. Several groups of microorganisms are involved in the degradation of air pollutants in biofilters, including bacteria, actinomycetes, and fungi (Leson and Winer 1991).

Data presented in the study demonstrated that when microbial population are acclimated and maintained, they can achieve the approximate 87% degradation of MCB. This shows that the use of coal as a packing media for higher growth of bacteria in the TBAB inoculation with municipal activated sludge reliable, efficient and easy to operate and maintain. Elimination capacities of MCB increased with an increase of the influent VOC loading, but removal efficiencies decreased with the increase in inlet load, which is probably due to the substrate inhibition effects at high MCB concentration. After starvation, the TBAB lost its ability to degrade MCB initially period but recovered very quickly. MCB concentration profile along the bed height indicated that the bottom section of TBAB has the best performance for all concentrations. The $r_{\text{max}}$ and saturation constant $K_m$ was to be found as 1.304 g m$^{-3}$ s$^{-1}$ and 113.446 g m$^{-3}$, respectively. It is our view that the information contained herein shall be useful for designing of coal based TBAB for the biodegradation of MCB.

Acknowledgments
Facilities provided by IIT, Roorkee and financial support provided by Ministry of Human Resource Development, Government of India is greatly acknowledge.

References
Biodegradation of mono-chlorobenzene in biofilter


