Biofiltration technology for the removal of toluene from polluted air using *Streptomyces griseus*

Elham F. Mohamed[a], Gamal Awad[b], Caroline Andriantsiferana[c] and Ahmed I. El-Diwany[b]

[a]Air Pollution Department, Environmental Research Division, National Research Centre, Giza, Egypt; [b]Chemistry of Natural and Microbial Products Department, Division of Pharmaceutical industries, National Research Centre, Giza, Egypt; [c]Laboratory of Chemical Engineering, University of Toulouse, ENSIACET, Toulouse, Cedex, France

ABSTRACT

Biofiltration technology has been recognized as a promising biotechnology for treating the volatile organic compounds (VOCs) present in polluted air. This study aims to investigate the performance of a biofiltration system of *Streptomyces griseus* sp. DSM-40759 immobilized on activated carbon (PICA S23) towards the adsorption and degradation of toluene vapour as well as to regenerate the activated carbon *in situ*. The batch studies were performed using nutrient agar medium and basal salt medium (BSM) for microbial growth. Initially the pre-cultures were incubated at a temperature of 28°C on a rotary shaker at 150 rpm. After two days, the strain *S. griseus* DSM-40759 was immobilized on a known weight of activated carbon (12 g). The results of biofilter performance showed three different stages with a quick adsorption phase with approximately 95% of toluene removal after 70 min, a slow biotransformation phase by immobilized cells. In the later, the removal efficiency decreased significantly with the extension of time and reached 60% during this stage. Moreover, a final quick removal phase by the immobilized cells had an average removal efficiency of toluene around 95% after 500 min. The toluene degradation was found to be more than 84% after the second cycle and the biofilter was still capable of removing additional toluene. Thus, the results demonstrated the feasibility and reusability of a new biofilter system for toluene removal as well as extending the activated carbon's capacity and this could be a potential solution to reuse the activated carbon in industrial application.

1. Introduction

Air pollution is a complex problem involving particles, asbestos, gaseous contaminants and volatile organic compounds (VOCs). VOC emission is one of the cores of atmospheric pollution.[1] In ambient air, VOC is associated with emissions from a range of sources such as motor vehicle exhaust, petroleum refineries, plastics, synthetic resins and use of solvents.[2,3] Most of the VOCs such as benzene and toluene are toxic and carcinogenic substances. Toluene is widely used in the chemical industry and many applications. Even at low concentrations, toluene has been found to be carcinogenic, causes damage to the liver and kidney, has adverse effects on the central nervous system and causes genetic damage.[4]

Traditional technologies commonly used for indoor air pollution control are thermal oxidation, photocatalytic oxidization, incineration, condensation and adsorption.[5–8] However, these methods are expensive due to the more energy requirements, risks to produce harmful secondary compounds that require further treatment since they simply convert target material into another phase and low removal efficiency and by-product.[9,10]

As an alternative, biological processes are emerging as an attractive alternative approach to remove volatile contaminants, odours or other hazardous particles present in gaseous waste streams.[11,12] Among the biological treatment methods, biofiltration has attracted considerable interest in the last few years.[13] Biofiltration has been recognized as a promising alternative and environmentally friendly biological technology for treating the organic and inorganic pollutants present in waste gases and wastewater as compared to the conventional treatment technologies.[14,15] The important advantages of biofiltration for waste gas treatment are (1) less expensive to build and operate, (2) operating at room temperature, (3) effective at low concentrations and high flow rates, (4) effective at high humidity levels, (5) high removal efficiency and (6) 'green' technology which does not use chemicals and does not produce wastes potentially dangerous for the environment.[16]

Several different packing materials have been used in biofiltration as support media for biomass immobilization such as activated carbon, soil, wooden chips, peat, compost and ceramics.[17]

Activated carbon was the most widely used adsorbent in air and water pollution control due to its excellent adsorption abilities for different pollutants. Biologically activated carbon (BAC) has been showing the ability to remove gaseous contaminants in biofiltration by a synergy of microbiological degradation and activated carbon adsorption.[18–20] Hence, combining adsorption and biofiltration processes is a very promising method for the treatment of VOCs.

The problem, although studies on VOC biodegradation in biofiltration have been conducted over the last years, many researches have been limited to knowledge and data on a particular VOC and many aspects of this process still remain unknown. Further, literature surveys revealed that only few researches actually deal with the regeneration of activated carbon as packing material *in situ* with immobilized bacteria.

The main objectives of the present work are (1) to develop a new economical and effective immobilized biofiltration system by immobilization of *streptomyces griseus* DSM-40759 onto activated carbon (PICA S23) as high superficial area packing medium, then (2) to investigate the performance of a lab-scale biofilter for the treatment of gaseous toluene and (3) to study the possibility of regeneration of activated carbon in this bioreactor and investigate the recovery of removal efficiency in the biofilter after the first cycle utilization, which it is a critical factor for evaluating the biofilter performance.

2. Materials and methods

2.1. *Microorganism and media composition*

Pure strain of *streptomyces griseus* DSM-40759 was used. Nutrient agar medium and basal salt medium (BSM) were used for microbial growth. Nutrient agar medium contained 1 g beef extract, 2 g yeast extract, 5 g peptone, 5 g NaCl and 15 g agar in one litre distilled water. The composition of BSM was 1.5 g K_2HPO_4, 0.5 g KH_2PO_4, 0.5 g $(NH_4)_3PO_4$, 0.5 g NaCl, 3 g Na_2SO_4, 2 g yeast extract, 0.5 g glucose, 0.002 g $FeSO_4$ and 0.002 g $CaCl_2$ in 1L distilled water.

Initially, a fresh slant was prepared by using agar-agar and bacteriological agar. From a parent slant, a loop was struck on a freshly prepared slant and then the cells were allowed to grow by keeping the slant in an incubator at 30°C. Once the cells grew on the slant without any contamination, a liquid suspension culture was then prepared from this slant by striking a loop and adding it in the nutrient medium. Nutrient medium composition consisted of ammonium nitrate (1 g/L), ammonium sulphate (0.50 g/L), sodium chloride (0.50 g/L), di-potassium hydrogen orthophosphate (1.5 g/L), potassium di-hydrogen orthophosphate (0.5 g/L), ferrous sulphate (0.002 g/L), calcium chloride (0.01 g/L) and magnesium sulphate (0.50 g/L) in distilled water. Suspended biomass concentration was measured optically at 610 nm using T80 + UV/VIS spectrophotometers, PG Instrument Ltd., range: 190–1000 nm.

2.2. *Preparation of granular activated carbon*

The experiments were conducted with charcoal activated carbon (PICA S23). granular activated carbon (GAC) was washed several times with deionized water to remove carbon fines and dried in an oven at 105°C for 24 h, and then sterilized with autoclaving at 121°C for 15 min. The physical properties of AC S23 such as specific surface (m^2/g), microporous volume (cm^3/g), mesoporous volume (cm^3/g), pore size diameter (Å), volume weight mean diameter (μm), bulk density (kg m^{-3}), ash content (%), carbon content (%), pH $_{pZC}$, total acidic sites (mmol/g), total basic sites (mmol/g), water content (%), particle mean diameter (mm) and bed porosity are listed in Table 1.

2.3. *Microbial immobilization*

To measure the immobilization capacity of a support material, the batch studies were carried out in a flask. Initially the precultures were incubated at a temperature of 28°C on a rotary shaker at 150 rpm. After two days, they were harvested for immobilization. The strain *Streptomyces griseus* DSM-40759 was used for immobilization. The working volume was 100 mL in 250 mL Erlenmeyer flasks. A known weight of activated carbon (12 g) was used for the immobilization study (48 h). During immobilization, the flasks were kept in a refrigerator. The growth of suspended microorganisms was determined by measuring the optical density of the suspension at 610 nm. The quantity of immobilized microorganisms was calculated by the difference between cells in suspension before and after immobilization on the solids, that is, after two days of immobilization.[21] A control experiment without solids was also conducted.

2.4. *Scanning electron microscopy of activated carbon samples*

Scanning morphological characteristics of the AC surface before and after bacterial immobilization were examined with a Scanning Electron Microscope (Jeol JSM-840A, Japan) to ascertain the cell immobilization.

Table 1. Characterization of the activated carbon used.

Parameters	S23 PICA
Origin	Coconut
Specific surface (m^2/g)	1230
Microporous volume (cm^3/g)	0.49
Mesoporous volume (cm^3/g)	0.04
Pore size diameter (Å)	17
Volume weight mean diameter : d_{43} (Granulometric analysis, µm)	447
Bulk density (g/L)	1013
Total acidic sites (mmol/g)	0.3
Total basic sites (mmol/g)	0.98
Particle mean diameter (mm)	$0.8 \leq pd \leq 1$
Water content (%)	3.9
Bed porosity	0.3
Carbon content (%)	92.28
Ash content (%)	3.2
pH_{pZC}	9.7

2.5. *Biofiltration system and conditions*

Figure 1 shows the schematic diagram of the bench-scale biofilter system designed for this study. To compare toluene removal by biofilter packed with GAC (PICA S23), a cylinder-type stainless steel column biofilter (1.4 cm internal diameter × 24 cm height) was built. The biofilter bed was packed with *S. griseus* DSM-40759 immobilized filter material (12 g of activated carbon). The ratio between column diameter to GAC particle diameter was 14:17, the ratio above 10 recommended to avoid the wall effects.

The experimental set-up for continuous biofiltration consisted of nutrient reservoirs and a nutrient pump to supply the bacteria with nutrients and to prevent the packing material from drying; a liquid toluene storage bottle; a pump of air to evaporate toluene through a volatilization chamber before entering the biofilter; an air flow meter and finally inlet and outlet sample ports. The toluene vapour concentration was obtained by adjusting the flow rate. Air was blown with the aid of a pump through the waste gas generating unit containing liquid toluene operated at a fixed flow rate (2 L/min) at room temperature (25°C) with empty bed retention times (EBRTs) of 80s.

2.6. *Experimental procedure*

The experiments were carried out in three different steps with the same reactor, all at room temperature (25°C). In the first step, an empty reactor without microorganism or activated carbon was set up as a control reactor to evaluate the inlet toluene concentration in the experimental system. In the second step, the adsorption tests were initiated by placing GAC without attachment to bacteria in the reactor to investigate the AC performance towards the removal of toluene vapour in the reactor system. The virgin activated carbon was suspended in water to mimic the conditions of the treated filter. In the third step, a reactor was packed with *S. griseous* DSM-40759 immobilized AC filter material. The second cycle was carried out for materials (AC and BAC) to investigate the effect of bio-regeneration of the activated carbon. At regular time intervals, the samples of the waste gas from the inlet and outlet of the biofilter were collected and dissolved in 90% methanol and then analysed by high-performance liquid chromatography (HPLC). Toluene degradation in the biofilter was evaluated by measuring the inlet and outlet concentrations of toluene. Since toluene was a saturated liquid, the vaporization concentration could be controlled by adjusting volatile surfaces. As air flow and the ambient temperature remain unchanged, the concentration tends to be constant when the volatile surface of the liquid is constant. The performance of the bioreactor was evaluated by a series of parameters, such as the residual toluene concentration (mg/L) and the removal efficiency; and the fraction of the pollutant removed is expressed as (%).

2.7. *Sampling and HPLC analysis*

The air samples were trapped and collected from the inlet and outlet streams of the bioreactor using 90% methanol and analysed for toluene concentration using HPLC. The HPLC analysis was performed on clarity chromatography data systems. The HPLC system consisted of two pressure pumps (Sykam S1122 delivery system, Germany), the injection port with a 2 mL loop (Sykam S 5111 Injector valve bracket, Germany), a UV detector (Jasco UV-2070 Plus, Intelkigent UV/visible, Japan). For chromatographic separation, a C-18 semi-preparative column (Kromasil, 5 µm, 250 × 4.6 mm) was used. Methanol and water (90:10, v/v) were used as the mobile phase and the flow rate was adjusted to 1 ml/min. Sample volume (20 µL) was injected with the help of a micro-syringe, the runtime was adjusted to 10 min and UV absorbance was determined at 254 nm. Autochrom 3000 software was the data acquisition system. The calibration curve for toluene determination was made using different concentrations of toluene authentic sample (Sigma). The results obtained from the HPLC analysis of the samples were calculated using the above-mentioned calibration curve.

3. Results and discussion

3.1. *Morphological analysis of the activated carbon*

The top surface images of the activated carbon samples were analysed with scanning electron microscopy (SEM)

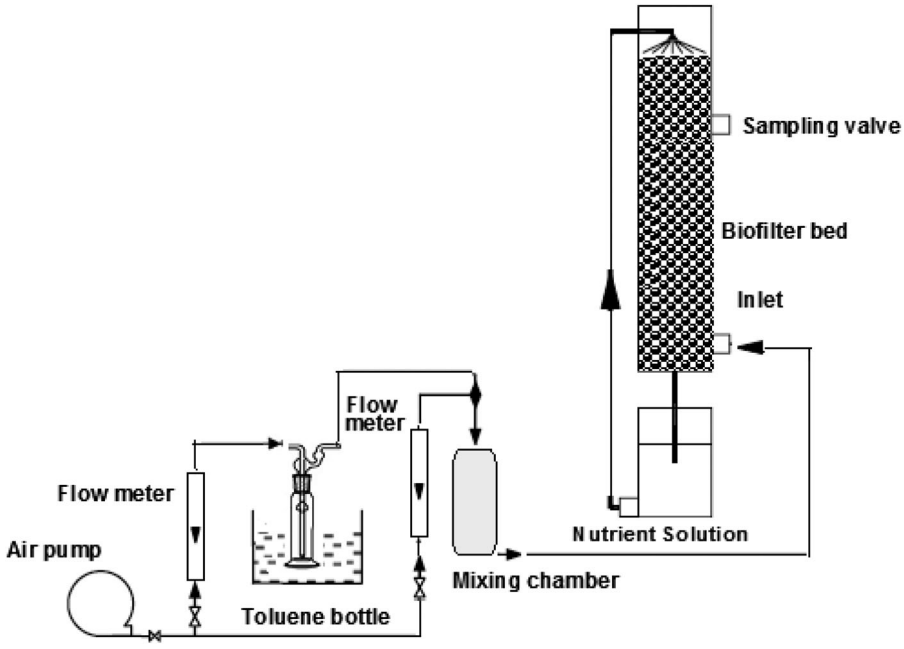

Figure 1. Schematic diagram of the laboratory-scale biofilter system for treatment of air containing toluene.

before and after cell immobilization in order to compare the changes in the surfaces. Figure 2 depicts the microscopic images of GAC before (a) and after (b) the immobilization of microbes at three different magnifications X1500, X2000 and X2500. As it is clearly shown in Figure 2(a), the activated carbon sample possesses a strong porous structure which consists of large pores and small pores. The SEM results (Figure 2(b)) illustrated that *S. griseus* DSM-40759 was successfully bound to the surface of AC. Scattered colonies of uniform, short rod-shaped bacteria were observed along the surface of the activated carbon. The quantity of immobilized *S. griseus* DSM-40759 cells onto GAC was 5.76 mg/g of GAC. The cells were both directly attached to the surfaces of the GAC and entrapped within the pore spaces of the GAC, thus minimizing the exterior sorption sites to the toluene vapours. There might be two reasons for the attachment of bacterial cells, including the physical effect between the cells and the activated carbon, and the adhesive effect between the activated carbon and the extracellular secretion of the strain.

3.2. *Control studies*

The main concern in the biological treatment of gas components is their chemical adsorption on the supporting material. Control studies were performed to measure the extent of toluene adsorption on the activated carbon supporting material. This was accomplished by measuring the differences in toluene concentration between the inlets in which toluene vapour was fed to the bottom of the empty reactor at constant flow rates of 2 L/min and the outlet of the reactor filled with activated carbon, in the absence of microorganisms. The reduction in toluene concentration of the outlet gas due to adsorption on the activated carbon and the inlet toluene curve versus time are presented in Figure 3. It was noticed from the figure that the toluene disappearance started very fast at the beginning of adsorption and then decreased. It can be seen that toluene concentration reached 143.7 mg/L within 500 min.

3.3. *Performance of the biofiltration system*

The performance of *S. griseus* DSM-40759 attached to the AC biofiltration system is shown in Figure 4. The figure illustrates the removal of toluene vapour for both activated carbons with and without the attached strain (previously discussed) for an experimental duration of 500 min, besides the inlet toluene curve.

The toluene concentration declined instantaneously to show higher fast adsorption in the virgin activated carbon than the case of with bacterial attachment. It was noticed that the toluene adsorption capacity of a virgin granular AC was about 10–20% higher than that of activated carbon with the attached microorganism in the biofilter. This indicates that initial adsorption was inhibited by the *Streptomyces* species attached to the exterior sites of the activated carbon surface. This may be explained by the assumption that some sites of AC in the media were not in contact with the toluene vapour. This decrease in the available number of

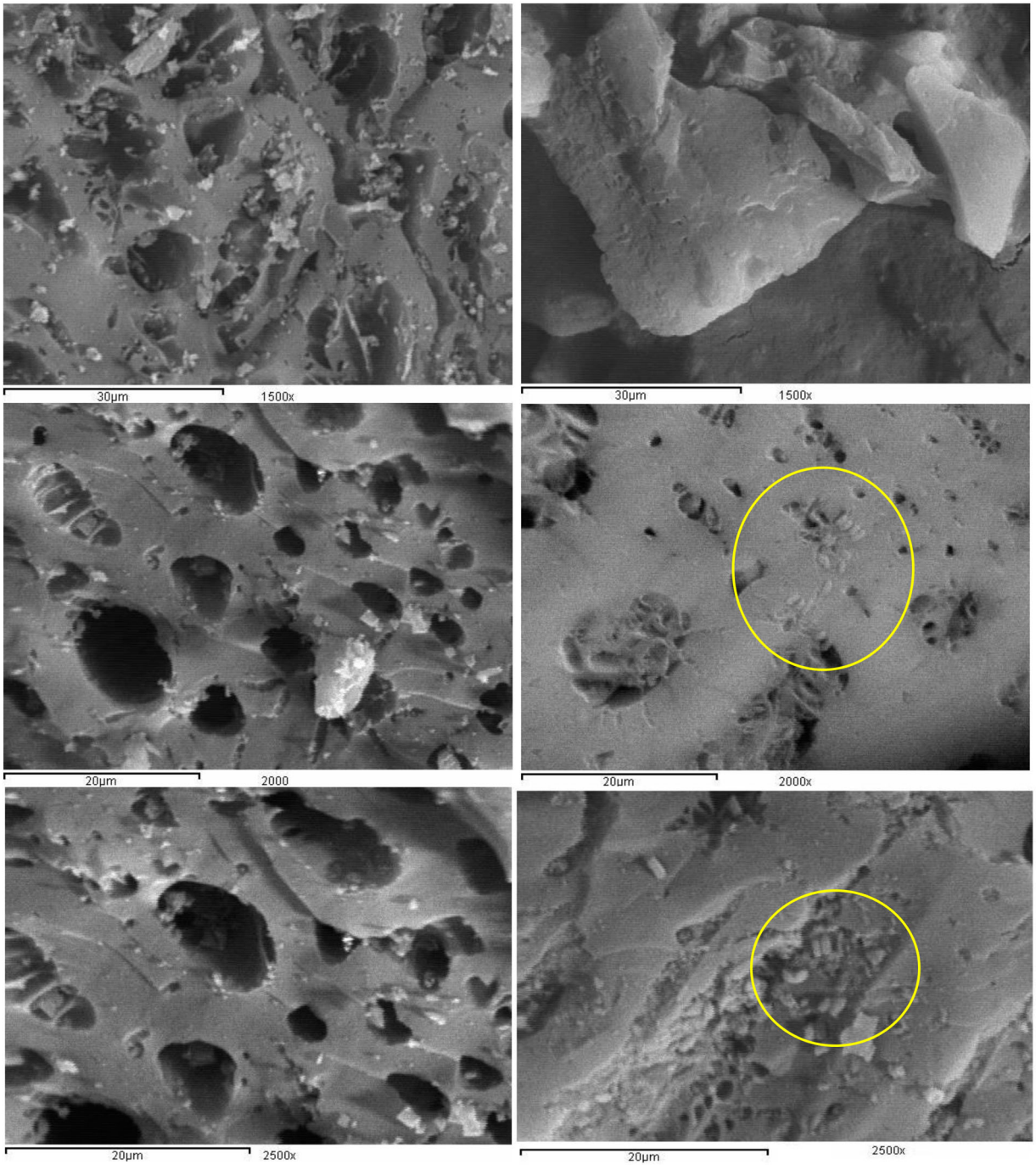

Figure 2. SEM of the original activated carbon (A) and the activated carbon with attached bacteria (B) (magnifications: 1500 ×; 2000 × and 2500 ×).

adsorption sites seems to be due to the blocking of access to some of them by the microorganism attached to the surface of the activated carbon. These results were well supported by the SEM analyses (discussed earlier in Figure 2(b)), where *Streptomyces* species were attached to the surface of activated carbon. The image of carbon surface structure confirmed the presence of *S. griseus* DSM-40759 on the carbon surface and thus minimizing of the exterior sorption sites to the toluene vapour. It is noted that *S. griseus* DSM-40759 used in this study has a size of approximately 0.5–0.8 nm and could be easily attached or filled in the microstructure of similar size. The negative effect of sorption by bacteria attached to the activated carbon is also cited in the literature [22,23] which found that phenol adsorption by AC was reduced due to the formation of biofilm of bacteria on the carbon surface. Additionally, Devinny et al. [24] and Dong et al. [25] investigated that a disadvantage

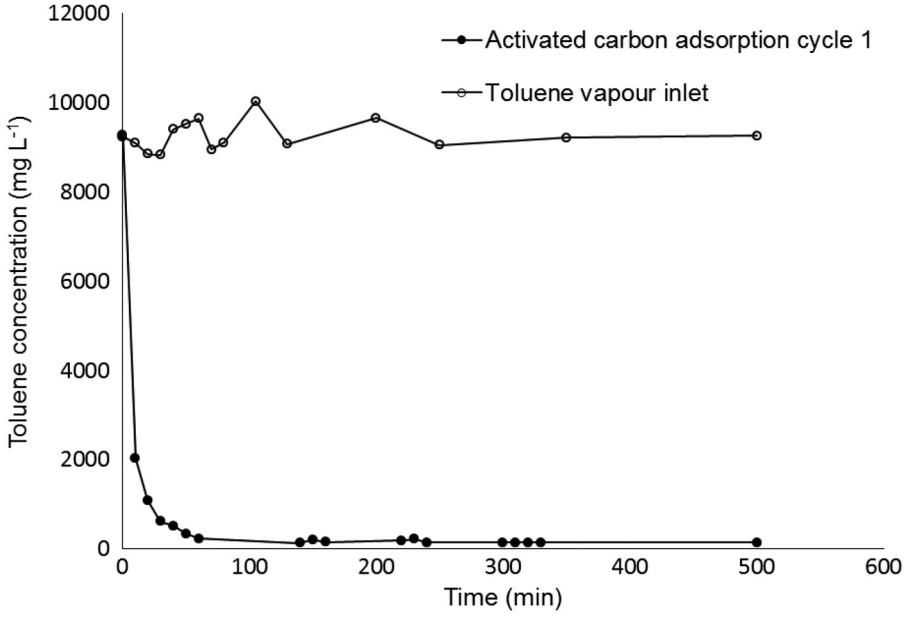

Figure 3. Kinetic adsorption of toluene using activated carbon before *Strptomyces griseus* DSM-40759 immobilization.

in using GAC in biofilters is that the adsorption capacity of activated carbon can be substantially reduced by water and microbial growth on its surface. It is remarkable that for the case of the virgin activated carbon without bacterial attachment, removal processes occur mostly in the form of rapid adsorption; however, for the activated carbon with bacterial attachment, synergy of both adsorption and biodegradation contributed to the removal of toluene.

Figure 4 shows the performance of the bioreactor, including the decrease in toluene vapour concentration during experimental time of 500 min. The biofilter performance shows three different stages: a quick adsorption phase, a slow biotransformation phase by immobilized cells and a final quick removal phase by the immobilized cells. In the first stage, toluene concentration dropped sharply. In the second stage, the concentration slightly increased and in the last stage, it dropped again. This performance can be explained as follows: the AC contains a highly developed pore structure, so the initial decrease in toluene concentration in the stage I was due to the adsorption processes. The initial adsorption stage was of 70 min, during which toluene was removed from the gas phases by adsorption onto AC

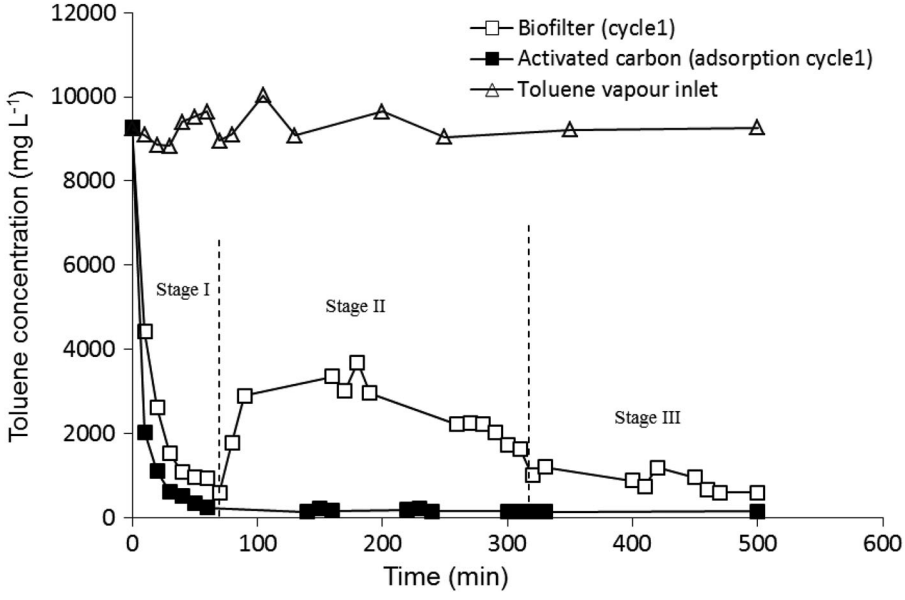

Figure 4. Comparison the performance of biofilter to that of the activated carbon without bacterial attachment towards the removal of toluene vapours inlet.

in the biofilter. During this period, the toluene vapour concentration was reduced from 9266 to 589 mg/L. This reduction in concentration was lesser than that observed with the virgin activated carbon, where the toluene concentration was reduced to 233 mg/L within 60 min, thus probably indicating that no biological oxidation of toluene vapour occurred during this stage and also that the change in the concentration was only due to the adsorption. The toluene substrate concentrations decreased to a stable level after the sorption phase; thus, the cells can easily grow with low substrate concentrations, resulting in better system performance for the removal of toluene vapour. The second stage indicates the growth period of microorganisms during which they get adjusted to the new environment and grow. During this stage, the bacteria strain was acclimated to toluene gas over approximately 130 min. In this second stage, the toluene concentration started increasing with the time due to the slow release of toluene to the microorganisms on the surface of the activated carbon. When the microorganisms are in the maximum growth phase, the biodegradation becomes predominant and the concentration gets reduced in stage III. At this third stage, the microorganisms might form a stable film on the GAC and also the organisms have sufficient contact time to release extracellular enzymes for the oxidation of organic pollutants present in an air stream. The microorganisms grow in a biofilm on the surface of activated carbon or are suspended in the biofilm surrounding the carbon particles, and remove the toxic compounds using a combination of physical adsorption and biological degradation. Moreover, this stage can be referred as the bio-regeneration phase of the activated carbon which is necessary for the system performance to remain stable. The same performance was reported by Dabhade et al. [18]; it was shown that phenol adsorption dominates at the initial stage followed by the growth period of microorganisms and increase in phenol concentration and in the last stage, biodegradation dominates after reaching the adsorption equilibrium.

The advantages of using a bacterial strain with AC as the packing material in biofilters were reported.[26,27] The immobilized cells were firstly protected from the excessive toxic action at high organic compound concentration compared with free bacterial cells. The other advantage was the decrease in the time required to start degrading organic pollutants at the same concentration than that with free cells.

3.4. *Toluene removal efficiency*

To investigate the system performance of the bioreactor, toluene waste gas removal efficiency was calculated and illustrated in Figure 5. In stage I, toluene was the sole pollutant, a quick start-up period was observed in the biofilter and approximately 95% of toluene could be removed within 70 min. The removal efficiency then decreased significantly with the extension of the operational time and reached to 60% during stage II. The average removal efficiency of toluene was then quickly increased to around 95% in stage III after 500 min (Figure 5). This performance may be due to the adsorption in the first stage; then desorption could occur due to a decreased toluene concentration in the contact phase near the exterior particle boundary. It follows that biodegradation could only occur in compounds that are both biodegradable and readily adsorbable. These results are in agreement with that obtained by Pandey et al. [28] who studied the biofilter for the treatment of waste gas containing diethyldisulphide for a period of more than two months, and the results of the performance indicated that diethyldisulphide removal efficiency was $94 \pm 5\%$. Babbitt et al. [29] also used activated carbon in biofilter to remove methanol from air, with a removal efficiency of 100%. The predominant conclusion was that the synergy effect is due to the simultaneous occurrence of well-developed biomass on the AC support, carbon adsorption and finally, biodegradation.

In this study, a removal efficiency of 95% was obtained for the inlet concentration of 9000 mg/L at an EBRT of 80s. Comparatively, Saravanan and Rajamohan [30] observed a maximum xylene removal efficiency of 50% for the inlet concentration of 1.2 g/m^3 at an EBRT of 88.2 s in a biofilter packed with press mud.

There is general agreement on the mechanisms of biofilters in the literature. It can be summarized as follows: a typical biofiltration process consists of two steps. Firstly, the contaminant is transferred from the air stream into liquid of the biofilm and adsorbed on a solid medium; then the pollutant is biodegraded by microbes living in the liquid phase or on the packing to carbon dioxide and water.[31,32] The microorganisms utilize these contaminants in two ways: (a) they contribute to the growth of new cellular material (anabolic pathway) and (b) they are sources of energy, which is synthesized through the respiratory system (catabolic pathway); ultimately converting the contaminants to biomass and metabolites.

3.5. *Bio-regeneration of activated carbon*

For the two reused systems, activated carbon without bacterial attachment (virgin carbon) and activated carbon with the immobilized bacteria (biofilter), the results are shown in Figures 6 and 7, respectively. Overall, the biofilter system showed the same removal

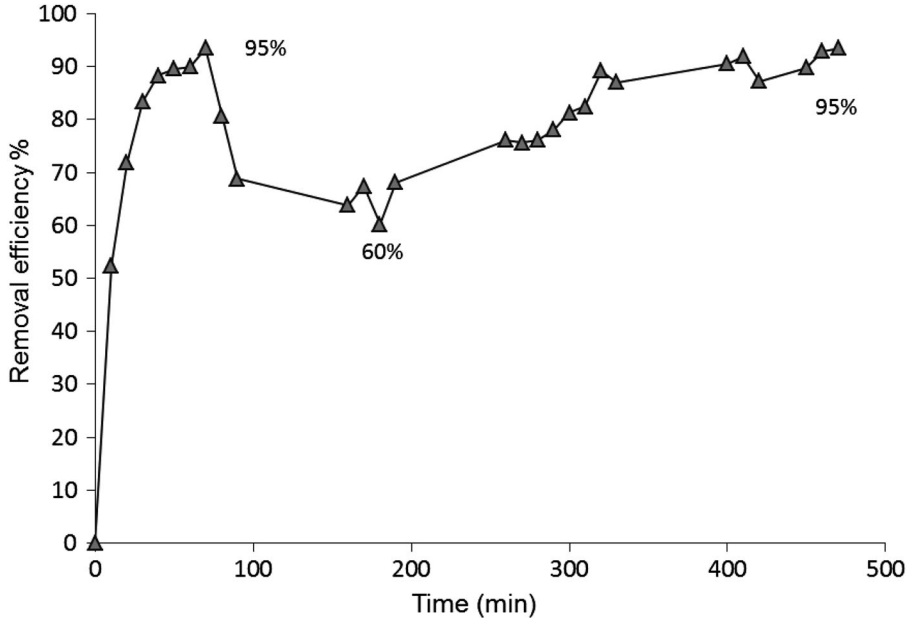

Figure 5. Toluene removal efficiency using the new biofilter.

profile (stages I, II and III) with good stability for the second experimental runs; however, the performance of the virgin activated carbon (without bacterial immobilization) deteriorated after the first run. In the case of 'virgin' activated carbon, inlet toluene concentration was about 9000 mg/L and after 500 min the toluene concentration was reduced to 150 mg/l in cycle 1. However, in cycle 2 with the same toluene inlet concentration (9000 mg/L), the activated carbon adsorbed about 24% of toluene and the concentration reached nearly 7000 mg/L. Thus, we can assume that the activated carbon was not completely saturated in the first cycle; so 500 min is not enough time to reach a steady state. For this reason, the concentration of toluene vapour in the outgoing gas did not increase in cycle 1 since the activated carbon was not completely saturated. On the other hand, the repeated operational reusing of the biofilter showed that the biofilter was still capable of removing additional toluene. Thus, the activated carbon could be regenerated by the *S. griseus* DSM-40759 bacterial cells, thus

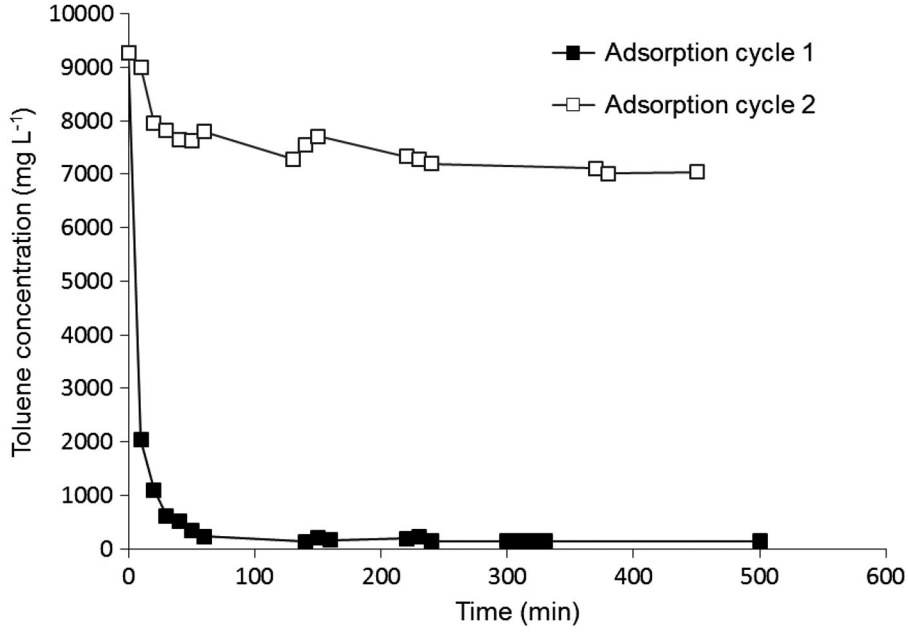

Figure 6. Kinetics of the toluene removal cycles onto virgin activated carbon (without bacteria attachment).

rendering the AC process amenable to long-term operation. The pattern of toluene contaminant removal during the second cycle (Figure 7) was essentially lesser than the mass adsorbed during the first cycle; this may be due to a portion of the contaminants remaining adsorbed on the carbon particles after the initial cycle. The same performance was investigated by Moe and Li [33] who found that the breakthrough curves for toluene adsorption during the second and third loading cycles were essentially identical; however, it was lesser than that obtained during the first cycle. Moreover, Sodha et al. [20] studied the regeneration of spent activated carbon by bacterial culture and found that the extent of activated carbon bio-regeneration was estimated to be 57.5%. These results indicate that it is possible to use the spent activated carbon as a bioreactor packing material; this could be a way for valorization of the activated carbon in industrial application. In the case of biofilter in the first cycle (stage I), the adsorption processes was predominant and toluene concentration reduced to about 600 mg/L. In stage II, the concentration was slightly increased due to toluene desorption and this stage can be considered as the acclimation period. In stage III, the biodegradation process was then predominant and the toluene concentration was then decreased; thus, both adsorption and biodegradation are two correlative processes which cannot be separated and the biofilter was still active during this period. This observation can explain why the toluene concentration did not increase with the continuous supply of toluene gas.

The result of the second cycle of the biofilter illustrated in Figure 7 revealed a quick start-up and worked well for the carbon at a short operational time. It was found that toluene vapour concentration sharply decreased to reach 6069 mg/L within 20 min in stage 1 (sorption stage). Moreover, the beginning of stage II shifted from 80 min to 30 min in the first and second cycles, respectively.

The results illustrated in Figure 8 indicated that the biofilter had removed toluene of more than 84% after the second cycle and the biofilter was still capable of removing additional toluene. However, the removal efficiency in the second cycle using activated carbon without bacterial attachment was dramatically decreased to 24%. The weak removal percentage of the second cycle with the virgin activated carbon may be due to the AC not being fully saturated in the first adsorption cycle; but in the case of AC with bacterial attachment, the significant removal percentage is an evidence of bio-regeneration. The lesser decline in the removal percentage in biofilter could be explained by chemisorption as the likely bonding mechanism, with irreversible chemical reactions occurring between the functional groups of the organic compound and the chemically activated surface of the carbon, thus resulting in the decrease in the adsorption surface area and loss of AC porosity. However, the results predominately proved that the new biofilter is a promising system for toluene removal, as well as a good process for activated carbon regeneration *in situ*, and thus is of greater importance than the adsorption surface area or other similar expressions of adsorption capacity. In the biofilter system, bacteria could regenerate the surface of the spent activated carbon using the adsorbed toluene as a source of food and energy.[34,35]

Two mechanisms account for explaining AC bio-regeneration. The first mechanism involves the biodegradation of organic compounds desorbed from the activated carbon. This theory includes desorption of the pre-adsorbed pollutants and biodegradation of the desorbed pollutants. As a consequence, the adsorbed organics are desorbed due to the concentration gradient between the activated carbon surface and system bulk. The results obtained by Olmstead and Weber [36] and Walker and Weatherly [37] support the hypothesis of adsorbent regeneration occurring by desorption of the pollutants from the pore structure to the biomass in the macropores of the adsorbent.

The second mechanism proposed by several investigators had hypothesized that bio-regeneration involves extracellular enzymes.[38,39] This theory supposes that some enzymes excreted by the bacteria diffuse into the biofilm, and the enzymes capable of penetrating into the mesopores of the AC and react with the adsorbed substrate. The weak adsorption affinity of the reaction products subsequently formed would result in eventual substrate desorption into the biofilm, where it would be further degraded.

Consequently, the performance of the bench-scale biofilter shows that the filter demonstrated a better performance than non-attached bacterial activated carbon as VOC adsorbent.

4. Conclusions

The results preliminarily proved that the new biofilter system composed of *Streptomyces griseus* DSM-40759 microorganism immobilized on the activated carbon (PICA S23) has an excellent performance in toluene removal. *S. griseus* DSM-40759 was successfully bound to the surface of AC; cells were both directly attached to the surfaces of the GAC and entrapped within the pore spaces of the GAC. Microorganisms immobilized on the activated carbon were capable of extending the activated carbon's capacity and lifespan. Moreover, spent activated carbon might be reused and

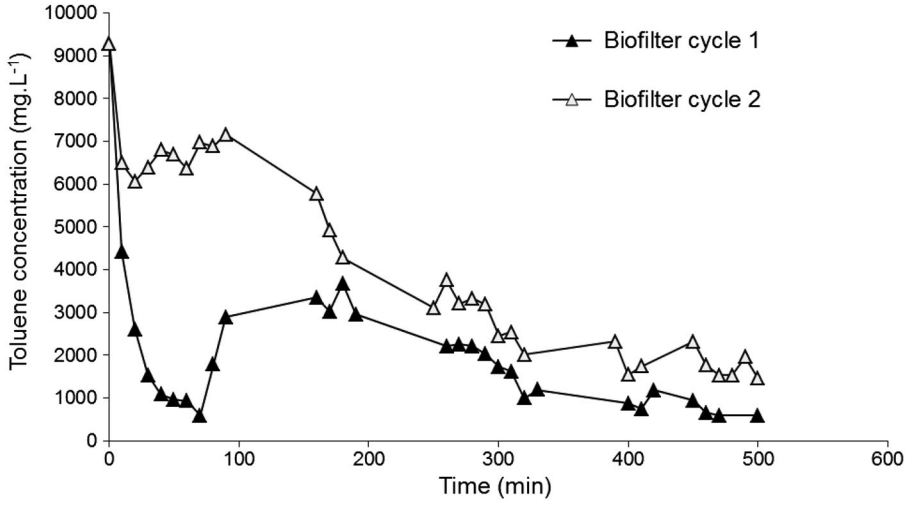

Figure 7. Kinetics of the toluene removal cycles using the biofilter.

bio-regenerated by immobilized microorganisms. The microorganism *S. griseus* DSM-40759 regenerates the surface of the spent activated carbon using the adsorbed toluene as a substrate. This could be a potential solution to reuse the activated carbon in industrial applications. The structure of the biofilm protects the microorganisms from difficult environmental conditions and retains the biomass inside the process, even when conditions are not optimal for its growth. The high adsorptive capacity provides improved production of the biofilm by limiting microbial inhibition from toxic contaminants while increasing contaminant removal efficiencies, especially during treatment start-up. The activated carbon also improves bio-system response to widely varying contaminants with high concentrations. Because microorganisms are retained within the biofilm, biofiltration allows the development of microorganisms with relatively low specific growth rates. Operating costs are usually considerably less than the costs of traditional technology, and the final treatment result is less influenced by biomass separation since the biomass concentration at the effluent is much lower than that for suspended biomass processes. Hence, this biofilter is a promising technique for pollutant removal by coupling both the adsorption process onto activated carbon and microbiological oxidation.

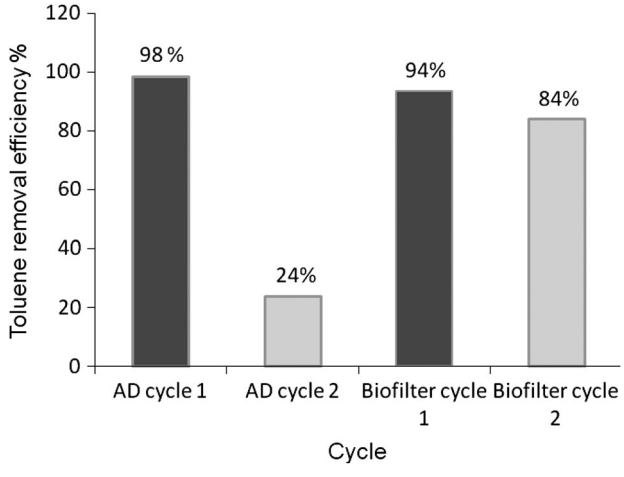

Figure 8. Toluene removal efficiency by using the new biofilter.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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