Removal of Ethyl Acetate from the Contaminated Air Stream in a Biofilter with the Active Biofilm of Pseudomonas putida

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Abstract: Active biofilm of Pseudomonas putida was developed in a biofilter for the removal of ethyl acetate from the contaminated air stream. Initially, in a separate batch experiment was conducted with 1.5g/l of ethyl acetate, to observe the biodegradability of ethyl acetate in an aqueous media. About 85% of ethyl acetate was biologically degraded with feed contained 1g/l ethyl acetate. Kinetic parameters for logistic model were defined to evaluate the growth behavior of the biofilm exist on the natural packing. The fabricated biofilter column was continuously operated in 25 to 65°C with the contaminated air with ethyl acetate from 400 to 5100 ppm and the inlet flow rate was varied from 0.12 to 1.5 cm³/min. The maximum removal efficiency of ethyl acetate from the air stream with low concentration of ethyl acetate (400 ppm) was 99%. The maximum elimination capacity of the fabricated biofilter for ethyl acetate was 412 g/m³.h. The results showed that the biofilter loaded with the active biofilm of P. putida had great capacity for the removal of ethyl acetate from the contaminated air stream.

Key words: Ethyl acetate · Biofilm · Pseudomonas putida · VOCs · Biodegradation · Logistic model

INTRODUCTION

Volatile organic compounds (VOCs) are frequently present in the exhaust air streams of many chemical plants and paint industries [1]. The organic contaminants may be present as air emission and the moist may contain toxic compounds. The generated VOCs may cause cancer or other serious health hazards and adverse environmental effects [2]. Ethyl acetate is one of the volatile organic compounds which is widely used in many chemical industries. Annually, large volumes of acetate compounds are released into the atmosphere during the manufacturing process that is endangering the air quality and public health [3].

Technologies available for the removals of VOCs are based on their nature in physical methods (absorption, adsorption), chemical techniques (incineration, oxidation) and biological processes such as biofiltration, biofilm trickling bed filters and bioscrubbers. The removal of VOCs from a polluted air stream using a biological process is highly efficient and more effective than those traditional methods and also has low installation and operation and maintenance costs [4,5].

In biofiltration the contaminated air passes through a packed bed fixed-film bioreactor. As air moves though the bioreactor, contaminants are transferred from the air stream into a biofilm fixed on solid support, as the organisms are grown on a fixed immobilized layer on the filter medium. Once the VOCs reached the biofilm, the microorganisms biodegrade the contaminants, adsorbed by the biofilm and the end products are harmless carbon dioxide, water and additional biomass [6,7].

The majority of biofilters reported in the literature for the treatment of VOCs have been operated nearly at neutral pH [6]. The moisture of packing materials in the biofilter is the effective parameter for ensuring the viability of the biofilm. It has been reported that the majority of the problem related to biofilter was caused by poor humidity control [8]. The optimal water content on a weight basis is 40-60% for most of the packing materials [8-10].

The elimination capacity (EC) of a biofilter presents the performances and ability of the active biolayer to handle high organic loaded air stream. The EC may increases with an increase of inlet load until it reaches an asymptote value. The maximum EC is determined by the biodegradability of the compound and the availability of oxygen to microorganisms attached to the filter medium [11].

Removal of ethyl acetate from waste gas by a trickle-bed air biofilter was evaluated. It was reported that the elimination capacity increased with an increase
in influent loading [12]. The maximum elimination capacity of ethyl acetate in the compost-wood and compost-polystyrene biofilter was 200 and 300 g/m^2.h., respectively. The compost-polystyrene biofilter had greater elimination capacity because the medium had higher porosity and larger active surface [13]. In an actual case, a gas-liquid-solid three phases flow airlift loop bioreactor activated sludge was applied to treat the air streams contained a mixture of ethyl acetate and ethanol [14].

The purpose of present research work was to demonstrate the biodegradation of ethyl acetate in batch and continuous for the fabricated biofilter. A kinetic model was defined to predict the cell growth in batch experiment. Also the capacity of VOCs elimination was studied. The removal efficiency of VOCs for various inlet ethyl acetate concentrations was also investigated.

MATERIALS AND METHODS

VOC: Ethyl acetate is one of the volatile organic compounds which is widely used in number of chemical industries. Acetate compounds are high-priority toxic chemicals and listed as highly pollutants [15]. In the color printing works, ethyl acetate is among the key pollutants included in exhaust air. Ethyl acetate is a kind of irritative and explosive compound with fragrant odor, which is harmful to respiratory systems of mankind [4]. Thus, ethyl acetate was selected as a nomine of VOCs. Natural packing was used as filter media. The biofilter was packed with walnut shell as it was implemented for biofilm support to remove ethyl acetate from vapor phase of the contaminated air stream. Ethyl acetate was supplied by Merck (Darmstadt, Germany).

Microorganism Cultivation and Medium Preparation: Pseudomonas putida PTCC 1694 was supplied by Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. The medium used for batch and continuous experiments consist of: yeast extract 0.3 g/l, KH₂PO₄ 1.52 g/l, Na₂HPO₄ 2.44 g/l, (NH₄)₂SO₄ 0.5 g/l, MgSO₄·7H₂O 0.2 g/l, CaCl₂·2H₂O 0.05 g/l, Fe₃(SO₄)₉·H₂O 0.6 mg/l, ZnSO₄·7H₂O 0.2 mg/l, CuSO₄·5H₂O 0.2 mg/l, MnSO₄·H₂O 0.2 mg/l, CoSO₄·H₂O 0.2 mg/l. Before any experiment, P. putida was adapted to a media contained ethyl acetate. The bacteria were grown in a medium with ethyl acetate concentration of 1g/l. Culture screening was conducted and the purity of the isolated culture was often controlled by culturing organisms on petri-dish and gram stain.

Experimental Set-up: The biofilter was constructed from a cylindrical Plexiglas column, internal diameter of 6.2 cm and total height of 100 cm (L/D = 16). The biofilter column was filled with walnut shell. The column empty bed volume after the establishment of biofilm was about 1750 ml. Fig. 1 shows experimental set up used for the biofiltration of the contaminated air. The air flow rate was adjusted and the supplements without any organic source were used to ensure humidity of the filter media.

![Schematic diagram for VOCs removal from the contaminated air stream](image-url)
The gas and liquid with counter-current flow had perfect contact on the walnut shell as filter media.

**Biofiltration Experiments:** Biodegradation of ethyl acetate in batch experiments was performed in five 500ml conical flask containing 250ml of medium and ethyl acetate for a period of 36 hours. In each flask, ethyl acetate concentration was varied from 1 to 5g/l, with an increment of 1g/l. Inoculum of *P. putida* was introduced into flasks number one to five, as the media contained 1 to 5g/l ethyl acetate. Flasks were placed on an Incubator Shaker (Stuart, S1500 series, USA). It was set at 180rpm and 30°C to enhance oxygen transfer rate into the media, thus higher cell growth was obtained. To determine ethyl acetate and biomass concentration in the flasks, samples were taken periodically. In continuous biofiltration, in order to establish a fixed biofilm, the column was inoculated with 500ml of seed culture. The biofilm was fully established on the natural packing (walnut shell). Ethyl acetate was added to the substrate tank with a defined concentration. An air stream was bubbled through the organic solution. The air was used as carrier gas for organic contaminants. The contaminated air was passed through the filter bed. In continuous mode of operation the effect of inlet temperature concentration and flow rate variations were analyzed. Nutrients were supplied downward with a flow rate of 5ml/min, to maintain the moisture content of the biofilm. Continuous biodegradation was ensured in the biofilter with constant supply of the contaminated air. The inlet and outlet concentrations of the ethyl acetate were determined by GC analysis. In a separate experiment, the effect of temperature variation on the VOC removal efficiency was investigated. The inlet gas temperature in the range of 25 to 65°C and fixed ethyl acetate concentration of 5g/l were also investigated. In order to monitor the effect of ethyl acetate concentration on removal efficiency, ethyl acetate in the substrate tank was varied from 5 to 25g/l at constant temperature of 45°C. In the final stage, at 45°C and the optimum concentration of ethyl acetate (20 g/l), the effect of inlet flow rate variations from 120 to 1500 cm³/min was investigated. Fig. 2 shows the biofilm gradually formed on the surface of walnut shells. The photo plates a, b and c from the same segment of the biofilter show the biofilm thickness gradually developed after 1, 12 and 30 days of operation.

**Analytical Methods:** To determine the ethyl acetate concentration, gas samples were collected from the inlet and outlet ports of the biofilter using a gas-tight syringe (Hamilton CO., Reno, Nevada, USA). The samples were analyzed by GC. The GC column was packed with 3 % OV-101, 80/100 Chromosorb. The temperatures of the injector, oven and detector were 220, 200 and 250°C, respectively. Nitrogen with a flow rate of 30ml/min was used as carrier gas. Optical density was measured using a spectrophotometer (UNICO, 2100 series, USA) at wavelength of 420nm (OD₄₂₀nm) and the calibration curve was prepared. Cell dry weight was also determined using a cellulosic filter, 25mm diameter and 0.25 pore sizes (Whatman, USA). The biomass concentration in the batch process was calculated by a correlation exists between cell dry weight and optical density. In order to study on surface morphology of the walnut and biofilm, a gold layer coating machine, manufactured by Emi Tec Co. from England was used. The morphologies of the walnut and microorganism were observed by Scanning Electron Microscope (SEM). The samples were examined under SEM using VEGATESCO made in Czech Republic.

![Fig. 2: Biofilm developed on the surface of biofilter media in (a) 1st day, (b) 12th day, (c) 30th day](image-url)
RESULTS AND DISCUSSION

Batch experiments were conducted, ethyl acetate in the media with concentration of 1-5 g/l was studied. Fig. 3 presents the cell dry weight of P. putida grown on various ethyl acetate concentrations. At high concentration, removal of ethyl acetate by microorganism was faced to long lag phase. Probably, that was due to intoxication of bacteria with substrate inhibition for ethyl acetate at higher concentration. In low ethyl acetate concentrations (1 to 2g/l), the removal was observed with a short lag phase. The exponential phase was completed within 16 and 20h for 1 and 2g/l of ethyl acetate and then it was shifted to stationary phase.

![Graph showing cell dry weight of P. putida grown on various ethyl acetate concentrations.](image)

**Fig. 3: Cell dry weight of P. putida grown on various ethyl acetate concentrations**

The concentration profiles for ethyl acetate in batch experiment are shown in Fig. 4. Maximum ethyl acetate removal efficiency of 85% was obtained at low concentration of ethyl acetate (1g/l). As the ethyl acetate concentration in the media was increased to 5g/l, the removal efficiency was drastically dropped to 23% that was due to the substrate inhibition.

Theoretically cell growth rate is expressed in equation (1). Maltinus equation represents the exponential growth in a batch culture [16].

\[
\frac{dx}{dt} = \mu x
\]

(1)

Where \(x\) is the cell dry weight concentration (g/l) and \(\mu\) is the specific growth rate (h\(^{-1}\)). The plot for \(\frac{dx}{dt}\) vs \(x\) was fitted linearly and presented in Fig. 5. The specific growth rate can be obtained from the slope of the line drawn based on obtained experimental data. As the ethyl acetate concentration increased, the slope of the lines and the value for \(\mu\) gradually increased.

At high substrate concentration, the cell growth was inhibited. The cell growth rate was evaluated by a kinetic model. Logistic equation was a fair kinetic model for the prediction of growth curve. The specific growth rate predicted by Logistic model given by equation (2) [16].

\[
\mu = \mu_{max}(1 - \frac{x}{x_{max}})
\]

(2)

Where \(x_{max}\) is the maximum cell dry weight concentration (g/l). By substitution of equation (2) into equation (1) and performing integration, the following equation for the cell concentration was obtained [16]:

\[
x = \frac{x_{0} \exp(\mu_{max} t)}{1 - \left(\frac{x_{0}}{x_{max}}\right)(1 - \exp(\mu_{max} t))}
\]

(3)

Where \(x_{0}\) is the initial cell concentration after inoculation. The above equation was used to predict the cell growth in batch experiments. In this research, inoculation volumes were kept constant for batch experiments. The logistic model was a fair approximation of the growth curve. Matlab (V 7.4), computer software was used to define logistic growth kinetic parameters. The biodegradation of ethyl acetate at various concentrations have been determined using P. putida free cells. The kinetic parameters defined by logistic model for P. putida in batch experiments are summarized in Table 1.

In continuous mode of operation the effect of inlet temperature variations for inlet ethyl acetate concentration (5 g/l) were investigated. Fig. 6 shows the effect of temperature on ethyl acetate consumption. The removal efficiency (RE) for the biofilter for both cases, low and high temperature was high, that means temperature variation did not influence the biofilter's RE. At 45°C, ethyl acetate was completely utilized before 12 hours of operation. That means at low temperature ethyl acetate was last for 24 hours. This would prove that, the substrate consumption rate at high temperature was higher than that of at low temperature.

<table>
<thead>
<tr>
<th>Ethyl acetate concentration (g/l)</th>
<th>(x_{0})</th>
<th>(x_{max})</th>
<th>(\mu_{max})</th>
<th>(X_{0}/X_{max})</th>
<th>(R^{2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 g/l</td>
<td>0.027</td>
<td>0.049</td>
<td>0.336</td>
<td>0.032</td>
<td>0.994</td>
</tr>
<tr>
<td>2 g/l</td>
<td>0.027</td>
<td>0.079</td>
<td>0.235</td>
<td>0.031</td>
<td>0.995</td>
</tr>
<tr>
<td>3 g/l</td>
<td>0.027</td>
<td>0.103</td>
<td>0.111</td>
<td>0.028</td>
<td>0.999</td>
</tr>
<tr>
<td>4 g/l</td>
<td>0.027</td>
<td>0.094</td>
<td>0.093</td>
<td>0.029</td>
<td>0.998</td>
</tr>
<tr>
<td>5 g/l</td>
<td>0.027</td>
<td>1.247</td>
<td>0.071</td>
<td>0.021</td>
<td>0.969</td>
</tr>
</tbody>
</table>
Fig. 4: Ethyl acetate concentration profile in the batch experiment

Fig. 5: The rate of cell growth vs biomass concentration developed as biofilm

Fig. 6: Effect of temperature on ethyl acetate consumption in continuous mode

Fig. 7: Ethyl acetate concentration in continuous mode for various substrate concentrations

Fig. 8: Ethyl acetate concentration in continuous mode for various air flow rates

Fig. 9: Elimination capacity and removal efficiency of the biofilter as a function of inlet organic load (ethyl acetate)
Fig. 10: Scanning Electron Microscopy of the outer surface of walnut
(a) magnification of 500 (b) magnification of 1000

Fig. 11: Scanning Electron Microscopy of the outer surface of walnut with biofilm
(a) magnification of 2000, (b) magnification of 4000
In the next set of experiments, the effect of substrate concentration was investigated in the range of 5 to 25 g/l. Fig. 7 depicts the reduction of ethyl acetates at variable concentration (5-25g/l) in the biofilter with respect to time. The RE for ethyl acetate was high (99%) when the inlet load was low. The RE decreased to 87% by increasing the ethyl acetate concentration up to 25 g/l.

In the final stage of the experiments, the effect of air flow rates from 120 to 1500 cm³/min was investigated at constant temperature (45°C) and fixed concentration of ethyl acetate (20 g/l). Fig. 8 shows reduction of ethyl acetate with respect to time at various air flow rates. At low air flow rate, a high RE value of 94% was achieved, since there was sufficient time for biodegradation of ethyl acetate by the active biofilm. As the air flow rate increased, the rate of penetration of substrate (ethyl acetate) into the biofilm may be limited. That was caused by the limited mass transfer and also the residence time was shorter than the low air flow rate. The removal of ethyl acetate from air stream by the active biofilm was dropped. The RE decreased until it reached to 62% when the air flow rate was set at 1500 cm³/min (Fig. 8).

The data for EC and RE vs inlet organic load carried out by the contaminated air were illustrated in Fig. 9. The dashed line is the predicted value and the solid line represents the actual data. Generally, for a given pollutant, the EC increases with the increasing inlet organic load until it reaches an asymptote value. In this biofilter the EC was increased until 412 g/m² h, even though longer experiments are needed to verify it, if this is the actual maximum EC. In addition the RE was slightly decreased as the organic load was increased from 164 to 659 g/m² h.

In order to study on surface morphology of the walnut and biofilm, SEM was used. In this case, a layer of gold was coated on both the walnut pretreated with Sodium Hydroxide solution (NaOH) and the biofilm developed on the treated walnut surface. The SEM micrographs were taken from the samples. SEM micrographs are shown in Figs. 10 and 11. SEM micrographs show that the walnut treated with NaOH had uniform pores shapes with honey bee nest structures. The approximate sizes of these pores were from 10 to 30µm (Fig. 10). This porosity made the filter media a suitable environment for the growth and immobilization of \textit{P. putida}. Fig. 10 shows the environment the walnut was highly porous, uniform pore size with mean value of about 20 µm.

SEM micrographs of the biofilm also show that \textit{Bacillus} with the rod-shapes are belong to \textit{P. putida} were grown very well on the walnut pores (Fig. 11). According to the images, approximate length of \textit{P. putida} is 2 to 5µm.

CONCLUSION

It was concluded that \textit{P. putida} was very successful organism in all experiments with batch culture and continuous biofilter for the removal of ethyl acetate from the contaminated air stream. At low concentration of air contaminants the biofilm had 99% of RE, while the concentration of ethyl acetate increased the RE was also dropped. The EC of the fabricated biofilter was increased until 412 g/m² h. The active biofilm was loaded with all uniform rod shape of \textit{P. putida}. The biofilm gained more biocatalyst activities as the air stream temperature increased. The batch growth of \textit{P. putida} was projected by Logistic model and the experimental data were well fitted with the model as R² was about 0.99.

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REFERENCES


