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Performance and Kinetic Evaluation of Ethyl Acetate Biodegradation in a Biofilter Using *Pseudomonas Putida*

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Abstract: Biodegradation of ethyl acetate in batch and continuous cultures was investigated. *Pseudomonas putida* was selected as a biological agent to biodegrade ethyl acetate. In batch experiment, biodegradation of ethyl acetate was performed in the range of 25-35°C. Walnut shells treated with alkaline solution as natural packing materials provided an appropriate environment for the growth and immobilization of microorganisms. The active biofilm was fully established on the surface of natural packing material. In the early stage, experiments were performed at Empty Bed Resistance Time (EBRT) of 60 s. Maximum removal efficiency of 99% was achieved at inlet concentrations lower than 430 ppm of ethyl acetate. The removal efficiency dropped to 80% with an increase of inlet concentration of ethyl acetate. In order to enhance the removal efficiency, the EBRT was increased from 60 to 75 s. At EBRT of 75 s, the removal efficiency was maintained above 80% even though the inlet concentration increased to 5150 ppm. Michaelis-Menten and Logistic models were perfectly fitted to experimental data. In addition, the kinetic parameters of presented models were defined.

Key words: Biodegradation; Logistic model; Michaelis–Menten; Pseudomonas putida; Walnut shell.

INTRODUCTION

Volatile organic compounds (VOCs) which exist in exhaust air streams of chemical plants may cause serious health effects or unfavorable environmental problems [1, 2]. Acetate compounds are high-priority toxic chemicals. Ethyl acetate is an irritant and explosive which is widely used in chemical industries. Also, it is harmful to the respiratory organs [3, 4].

Current technologies for the VOCs removal are physical techniques [5, 6], chemical methods [7] and biological processes such as biofiltration, biotrickling bed filters and bioscrubbers. Biological process for the VOCs removal is highly efficient and more effective and economical than traditional methods [8–10].

In biofiltration, the polluted air passes through a packing media, pollutants are transferred from the air stream into a biofilm fixed on media support. Once the VOCs get in touch with the biofilm, the microorganisms biodegrade the contaminants; the end products are harmless products and additional biomass [11, 12].

There are several parameters that are important in performance of biofilter. The pH of medium is an effective factor for the growth of microorganism; neutral pH for growth of *P. putida* is reported in the literature [13]. The moisture of packing media is an effective parameter for ensuring the viability of the biofilm. The existing problems in the biofilter are caused by poor humidity control. Filter media moisture content of 40-60 wt.% for optimal biofilter performance has been reported [14, 15]. Also, packing material should have sufficient porosity and provide suitable environment for immobilization of microbial cells [16].

The aim of this work was to demonstrate the biodegradation of ethyl acetate by *Pseudomonas putida*. Optimum EBRT was defined and the removal efficiency of VOCs for different inlet ethyl acetate concentrations was determined. Michaelis-Menten and Logistic models were applied to predict the biochemical reaction rate in the process.

MATERIALS AND METHODS

Microorganism cultivation: *Pseudomonas putida* PTCC 1694 was supplied by Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. The medium consisted of: yeast extract 0.3 g/L, KH_2PO_4 1.52 g/L, Na_2HPO_4 2.44 g/L, $(NH_4)_2SO_4$ 0.5 g/L, $MgSO_4.7H_2O$ 0.2 g/L, $CaCl_2.2H_2O$ 0.05 g/L, $Fe_2(SO_4)_3.H_2O$ 0.6 mg/L, $ZnSO_4.7H_2O$ 0.2 mg/L, $CuSO_4.5H_2O$ 0.2 mg/L, $MnSO_4.5H_2O$ 0.2 mg/L, $CoSO_4.H_2O$ 0.2 mg/L.

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All chemicals were supplied by Merck (Darmstadt, Germany). Before any experiment, *P. putida* was adapted to a media contained ethyl acetate. The bacteria were grown in a medium with ethyl acetateconcentration of 1 g/L. The purity of the single strain culture was often controlled by culturing the organisms on petri-dish and gram stain. In order to establish a fixed biofilm, the column was inoculated with 500 mL of seed culture.

Biofilter set up: The biofilter was constructed from a cylindrical Plexiglas column with an internal diameter of 6.2 cm and total height of 100 cm. Walnut shell was used as filter media. To develop porosity and specific surface area of the packing media, walnut shells were soaked in a 0.5 M NaOH solution for 24 h. Treated walnut shells were sterilized at 121°C for 30 min and then washed with distilled water until the pH value of the discharge reached to neutral condition. After that, the biofilter was packed with walnut shell as an implement for supporting biofilm.

Biodegradation of ethyl acetate in batch experiments was carried out in a 500 mL conical flask containing 250 mL of medium and ethyl acetate. Inoculum of *P. putida* was introduced into flask, as the media contained 1 g/L ethyl acetate. Batch experiments were conducted in a temperature range of 25-35°C for a period of 36 hours. Flasks were shaken at 180rpm with an Incubator Shaker (Stuart, S1500 series, USA). To determine ethyl acetate and biomass concentration in the flasks, samples were periodically withdrawn.

The temperature of biofilter column was maintained at constant temperature (30°C) by means of a water jacket. The nutrient solution was sprinkled from the top of the column at a flow rate of 700 mL/day using a peristaltic pump (Thomas, model SR25 , Germany). In addition, sprinkling and circulating the medium maintained the required moisture content in the bed. Nutrients solution was replaced with fresh medium every 2–3 days. To ensure humidity of the filter media, the air flow was passed through a humidifier. Ethyl acetate was injected by means of a syringe pump (Harvard Apparatus, model NP 70-2208, USA) and vaporized into the humidified air stream. Pollutant concentration in the inlet air stream was controlled by adjusting ethyl acetate flow rates. The rate of inlet polluted air stream to the biofiter was controlled with a rotameter (Dwyer Instruments, Inc., USA).

The contaminated air and nutrient supplements flow into biofilter were in counter-current pattern while the contaminated air entered from the bottom of the column. The schematic diagram of the biofilter used for the removal of ethyl acetate from the contaminated air stream is shown in Fig. 1.

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 gas chromatograph (GC) (HP 7890, Hewlett–Packard, USA) equipped with flame ionization detector (FID). 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. Optical density was measured using a spectrophotometer (UNICO, 2100 series, USA) at wavelength of 420nm (OD_{420nm}) and the calibration curve was prepared. Cell dry weight was also determined using a cellulosic filter, 25mm diameter and 0.25 pore sizes (Wathman, USA). The biomass concentration in the batch process was calculated by a correlation exists between cell dry weight and optical density.

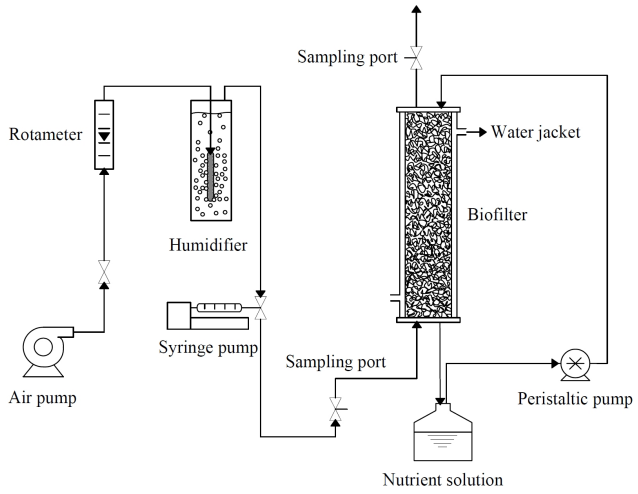

Fig. 1: Schematic diagram of the biofilter used for the removal of ethyl acetate

RESULT AND DISCUSSION

Batch experiments were carried out at different temperature of 25-35°C and ethyl acetate concentration of 1 g/L with a agitation rate of 180 rpm. Fig. 2 shows the cell dry weight of *P. putida* grown on various temperatures. The optimum growth rate of *P. putida* was reached at 30°C. The exponential phase was completed within 16 h at 30°C and then it was shifted to stationary phase.

In order to predict growth rate of *P. putida* logistic equation was applied. The specific growth rate predicted by Logistic model is given by equation (1) [17].

$$m = m_{\max}(1 - \frac{x}{x_{\max}}) \quad (1)$$

where x_{max} is the maximum cell dry weight concentration (g/L). By substitution of equation (1) into Monod equation and performing integration, the following equation for the cell concentration was obtained [17]:

$$x = \frac{x_0 \exp(m_{\max} t)}{1 - (\frac{x_0}{x_{\max}})(1 - \exp(m_{\max} t))} \quad (2)$$

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 sizes were kept constant for batch experiments. The logistic model was fairly approximated the growth curve. Matlab (V 7.4), computer software was used to define logistic growth kinetic parameters. The biodegradation of ethyl acetate at various temperatures 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.

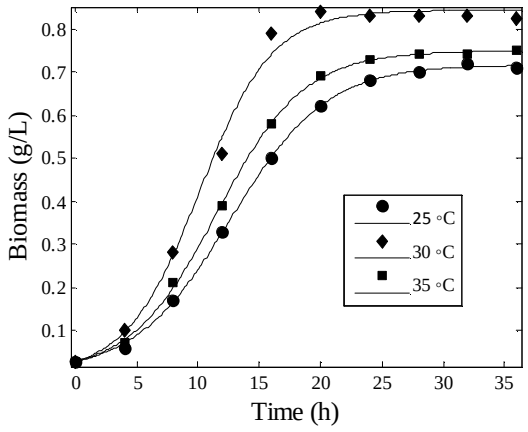

Fig. 2: Cell dry weight of *P. putida* at different temperature for ethyl acetate concentration of 1 g/L

Table1: Kinetic parameters of logistic model for biodegradation of ethyl acetate by *P. putida*

Temperature (30 ◦C)	X_0	X_{max}	μ_{max}	X_0/X_{max}	R^2
25	0.027	0.84	0.335	0.032	0.994
30	0.027	0.75	0.283	0.036	0.999
35	0.027	0.72	0.253	0.037	0.999

Prior to operation the organism was separately grown and packed column biofilter was sterilized and ready to operate. Once the biofilter reached to a high performance, then optimum operation condition needed to be identified. To determine optimum Empty Bed Residence Time (EBRT), biofiltration of ethyl acetate was performed at EBRTs of 15–75 s and ethyl acetate inlet concentration of 150 ppm. Effect of EBRT on the removal efficiency of ethyl acetate is shown in Fig. 3. At short EBRTs, since polluted air passes quickly through biofilter, contaminants have not sufficient time to be degraded with microorganisms; therefore the removal efficiency was not noticeable. When EBRT in the biofilter was increased, the removal efficiency of ethyl acetate increased to 99% and then it was quite stable. The removal efficiency reached to maximum value at EBRT of 60 s. Based on the desired EBRT (60 s) initial experimental runs were conducted.

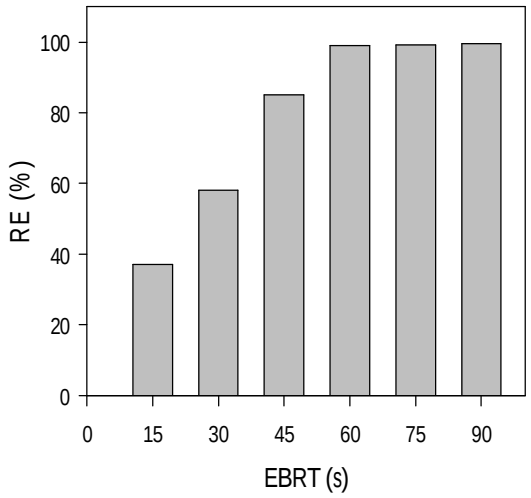

Fig. 3: Removal efficiency of ethyl acetate at different EBRTs (inlet concentration of 150 ppm)

After primarily experimental stage, sufficient bulk of biofilm formed on the solid surface of the walnut shells. The removal efficiency and inlet concentration of ethyl acetate are shown in Fig. 4.

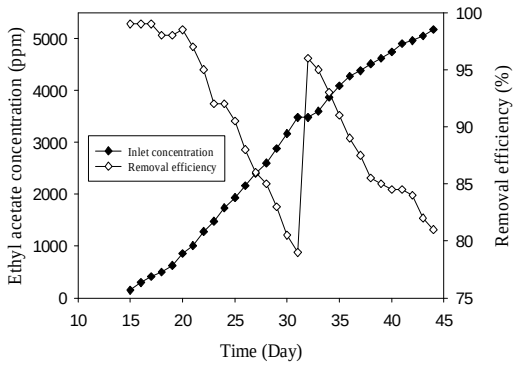

Fig. 4: Biofilter performance for ethyl acetate removal

Maximum removal efficiencies of 98–99% were achieved in the time interval of 15–20 days; while the inlet concentration of ethyl acetate was gradually increased from 150 to 860 ppm. During days of 20 to 30, the removal efficiency was maintained above 80% even though ethyl acetate concentrations in inlet stream were in the range of 1000–3150 ppm. The partially decrease in the removal efficiency of ethyl acetate might be due to an increase in inlet ethyl acetate concentration. There are many parameters seriously affecting the removal efficiency such as the residence time and biofilter's capability for the elimination of pollutants. In order to achieve desired removal efficiency at high inlet concentration, experimental runs were performed at EBRT of 75 s. The removal efficiency was more than 80% for the high inlet ethyl acetate concentration of 3470–5150 ppm.

Assuming that biodegradation rate defined by an enzymatic reaction, Michaelis-Menten equation represents the overall kinetic model in a biofilter.

$$r = \frac{v_m \times C}{K_s + C} \tag{3}$$

where r is the biodegradation rate, v_m is the maximum biodegradation rate, K_s is the half-saturation constant and C is pollutant concentration. In this model with the assumption of a steady-state condition, the following equation can be expressed.

$$r = -u_s \frac{dC_g}{dh} \tag{4}$$

where u_s is the gas superficial velocity and h is the height of biofilter. By substitution of equation (4) into equation (3) and performing integration, the following equation was obtained [18]:

$$\frac{C_{g,i} - C_{g,e}}{\ln (C_{g,e} / C_{g,i})} = v_m \left(\frac{q}{\ln (C_{g,e} / C_{g,i})} \right) - K_s \tag{5}$$

where θ is the contaminated air residence time. The kinetic constants were calculated from the slope and intercept of the lines drawn based on obtained experimental data for EBRTs of 60 and 75 s. The data presented in this model are shown with the same trends for two different EBRTs. The biokinetic constants (v_m, K_s) for EBRTs of 60 and 75 s were v_m= 61.05 ppm/s, K_s= 781.3 ppm and v_m= 72.42 ppm/s, K_s= 762.9 ppm, respectively. At long EBRT (75 s), pollutants had sufficient time to take part in the biochemical reaction; therefore, v_m increased with an increase in EBRT. Also, an increase in EBRT caused K_s to decrease; as a result the biodegradation rate increased.

CONCLUSION

In this work, biodegradation of ethyl acetate was investigated. Walnut shell was used as a natural packing material for biofilter media. *P. putida* consumed ethyl acetate as a carbon source, and the active biofilm of *P. putida* was developed on the surface of walnut shells. Experiments were carried out at EBRTs of 60 and 75 s. Maximum removal efficiency of 99% was achieved at inlet concentrations lower than 430 ppm of ethyl acetate. Also, removal efficiencies were greater than 80% under high loading conditions. Michaelis-Menten and Logistic models were well fitted to the experimental results and the kinetic parameters of these models were calculated.

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