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Removal of Acetone from Contaminated Air in Biofilter using *Pseudomonas putida*

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Abstract: Removal of acetone from polluted air was investigated using a biofilter. An active biofilm of *Pseudomonas putida* was used for the biodegradation of acetone in a column packed with walnut shell as biofilter. In continuous mode of operation, the effects of acetone concentration (50 to 200g/l) and inlet air temperature (25 to 55°C) on acetone removal efficiency were investigated. Maximum acetone removal efficiency of 80.5 percent was achieved, with acetone concentration of 50g/l. While acetone concentration increased to 200g/l, the removal efficiency dropped to 42 percent. The temperature variations from 25 to 55°C did not significantly influence the removal efficiency of acetone from the air stream. However, at high temperature acetone removal duration was shortened. A growth model for *P. putida,* based on acetone biodegradation was developed.

Key words: Acetone · Biofilter · Biodegradation · Polluted air stream · Logistic model

INTRODUCTION

Volatile organic compounds (VOCs) are frequently present in the exhaust air streams of petroleum refineries and chemical plants [1-4]. Their impacts on health and environment such as depletion of stratospheric ozone, ground level smog formation, odours and chronic toxicity are reported [5-8]. Most of these chemical compounds are considered as carcinogenic agents [9-11]. Therefore, cost effective technologies are needed to control emissions of VOCs and prevent discharge of toxic compounds into safe environment [12].

Over past decades, several technologies, such as incineration, catalytic oxidation, adsorption and biological treatment have been developed to control air pollution and prevention of VOCs emissions [13]. Recently, considerable attention has been paid to biofiltration as a cost effective and promising technology for purification of polluted air streams. The contaminated air with low concentrations of VOCs (50-400ppmv) is biologically degradable in a suitable process. Also, biofiltration is simple to operate and also has no negative environmental impact [14, 15]. Biofilter is basically a fixed-film bioreactor that provides a large contact area between the gas stream and the microbial film [16-18]. In this process, VOCs are present in the contaminated air stream and pass through a packed bed. The solid supports are covered with a biologically active layer [19]. As the contaminated air flows through the media and passes over the biofilm, VOCs transfer from the gas phase into the biolayer and penetrate into the solid phase. While the VOCs diffuse through active biolayer, organic matters are deteriorated and converted to harmless end products, such as carbon dioxide, water and biomass [20, 21]. The biodegradation of VOCs in aerobic process is carried out in two steps: oxidation of ketone functional group by molecular oxygen and then break down of the energized molecule to CO_2 and water [22].

For long duration of operation, especially with high VOCs organic load, biofilter may suffer from dryness of the filter bed, acidification and nutrient depletion that reduces microbial activity and declines the VOCs removal efficiency [23]. The efficiency of biofilter at high organic load is depended on biofilter performance and characteristics of the packing material, water content, pH of the media, supplementary nutrients and media temperature [14].

The objective of the present research was to evaluate the feasibility of the biofilter to handle high concentration of VOCs, using walnut shell as packing media, under crucial operating conditions. Walnut shell is a natural waste material which is locally available. It is normally used as a natural support for biofilm formation. Acetone was selected as chemical agent for the

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contaminated air stream. The removal efficiency of the biofilter to eliminate VOCs was investigated. Also, kinetic model for the growth of *P. putida* based on the acetone removal was developed.

MATERIALS AND METHODS

VOC: Acetone has widespread use in various industries. Its high vapor pressure, low boiling point (56.5°C), extremely high water solubility and high polarity characterized this compound to be easily emitted into environment [23]. Thus, acetone was selected as a nominee of VOCs. A biofilter packed with walnut shell was implemented to remove acetone from vapor phase of the contaminated air stream. Acetone was supplied by Merck (Darmstadt, Germany).

Microorganism Cultivation and Nutrients: *Pseudomonas putida* PTCC 1694 was supplied by Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. The microorganism was aerobically grown at 25°C and pH of 6.7 in nutrient broth. The initial media for seed culture consisted of yeast extract, peptone and glucose with concentrations of 1, 2 and 10g/l, respectively. The medium used for continuous experiments contained yeast extract 0.5g/l, peptone 0.3g/l, $MgSO_4$ 0.1g/l, NH_4Cl 0.5g/l, K_2HPO_4 0.4g/l, KH_2PO_4 0.1g/l, $Fe_2(SO_4)_3.H_2O$ 0.6mg/l and $ZnSO_4.7H_2O$ 0.2mg/l. Before any experiment, *P. putida* was adapted to a media contained acetone. The bacteria were grown in a medium with acetone concentration of 5g/l. The purity of the culture was monitored by growing the bacteria on Petri dish and gram stain.

Batch Experiments: Biodegradation of acetone in batch experiments was carried out in five 500 ml conical flask containing 200ml of medium and acetone for duration of 30 hours. Acetone concentration was different from 1 to 5g/l, with an increment of 1g/l in each flask. Inoculum of *P. putida* was introduced to the five flasks of media, containing 1 to 5g/l acetone. Flasks were placed on a hotplate magnetic stirrer equipped with temperature controller (VELP scientific, Italy). It was set at 100rpm and 25°C to enhance oxygen transfer rate into the media, thus higher cell growth was obtained. To determine acetone and biomass concentration in the flasks, samples were taken periodically.

Continuous Experimental Set up: The biofilter was constructed from a cylindrical Plexiglas column, internal diameter of 6.2cm and total height of 100cm (L/D 16). The biofilter was run in continuous mode and counter current flow operation. The pH of the medium was adjusted to 6.7 using diluted Hcl (0.1N). The media temperature was maintained at 25°C. The concentration of acetone in the gas phase was measured using a gas chromatograph (GC) (HP 5890, Series II, Hewlett–Packard, Palo Alto, CA) equipped with flame ionization detector (FID).

The experimental set up for continuous biofiltration consisted of diluted acetone and nutrient reservoirs, nutrient pump, steam bath, air blower, air flow meter, condenser and fixed film biofilter with inlet and outlet sample ports. The schematic diagram of the bench scale biofilter used for the biodegradation of VOC is shown in Figure 1. The biofilter column was packed with filter media. The bed medium was walnut shell, soaked in 1wt percent agar solution. The void volume of the column after establishment of the biofilm was 2156ml.

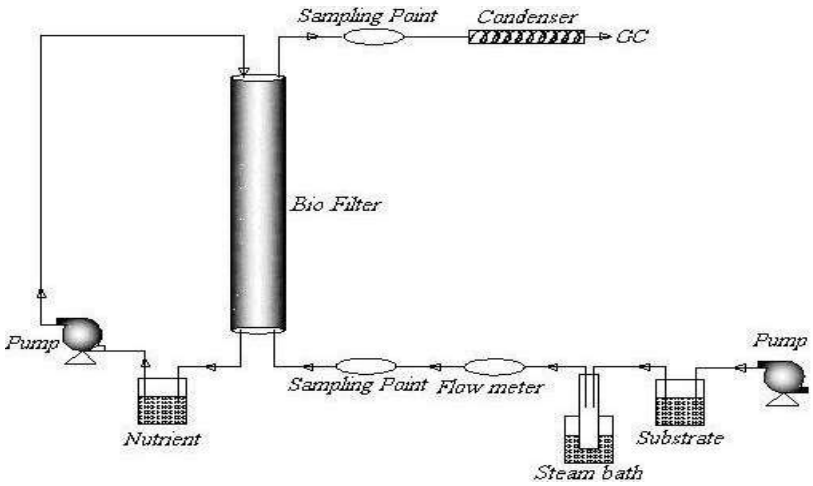

Fig. 1: Schematic diagram of the pilot scale biofiltration system

Fig. 2: Established biofilm on the surface of filter media from plate (a) to (e)

In order to establish biofilm, the column was inoculated with 400ml of seed culture. The biofilm was fully established on the natural packing. Acetone was added to the substrate tank with a defined concentration. Air was sparged through the organic solution. The air stream carried the organic contaminants. The contaminated air was passed through the filter bed. For acclimation of the microorganism with acetone, the reactor was continuously operated and gradually fed with acetone concentration of 10g/l. Then, acetone concentration stepwise increased to 50g/l.

Nutrients were supplied downward with a flow rate of 0.5ml/min, to maintain the moisture content of the biofilm. The nutrient mainly consisted of yeast extract and peptone with concentrations of 0.5 and 0.3g/l, respectively. Continuous biodegradation was ensured in the biofilter with constant supply of the contaminated air. The inlet and outlet concentrations of the organic compounds were determined by GC analysis.

In order to investigate the effect of acetone concentration on removal efficiency, acetone in the substrate tank was varied from 50 to 200g/l at constant temperature of 25°C. In a separate experiment, for observation the effect of temperature variation on the VOC removal efficiency, inlet gas temperature in the range of 25 to 55°C and fixed acetone concentration of 50g/l was investigated.

Figure 2 shows the biofilm gradually formed on the surface of walnut shells. The photo plates a, b, c, d and e from the same segment of the biofilter show the biofilm thickness gradually developed after 1, 3, 5, 7 and 8 days of operation.

Analytical Methods: To determine the acetone 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 percent 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 35ml/min was used as carrier gas.

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.

RESULTS AND DISCUSSION

Figure 3 depicts the variation of various acetone concentrations in batch experiment with respect to time at 25°C. Acetone at low concentrations was successfully removed from the air stream. There were quite similar trends for acetone reduction with respect to incubation time. Maximum acetone removal efficiency of 83 percent was achieved at low concentration of acetone (1g/l). As the acetone concentration in the media was increased to 5g/l, the removal efficiency was drastically decreased to 27 percent that was due to the substrate inhibition.

Figure 4 presents the cell dry weight of the microorganism in batch experiments. *P. putida* was successfully grown in media containing acetone as carbon source. At high concentration, removal of acetone by microorganism was faced to long lag phase. Probably, that was due to intoxication of the bacteria with acetone at higher concentration. In low acetone concentrations

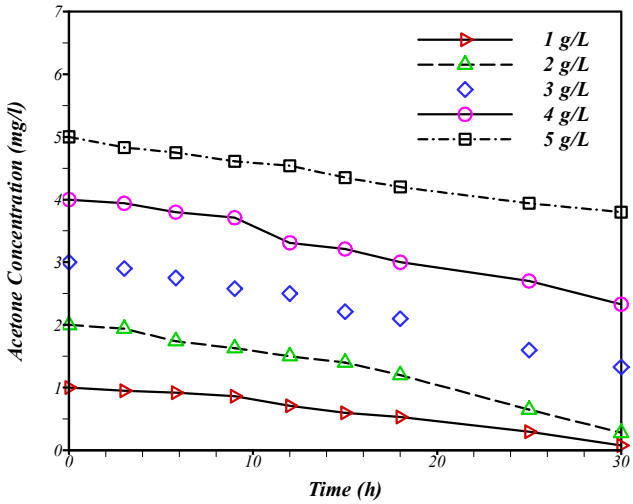

Fig. 3: Acetone concentration in the batch experiments

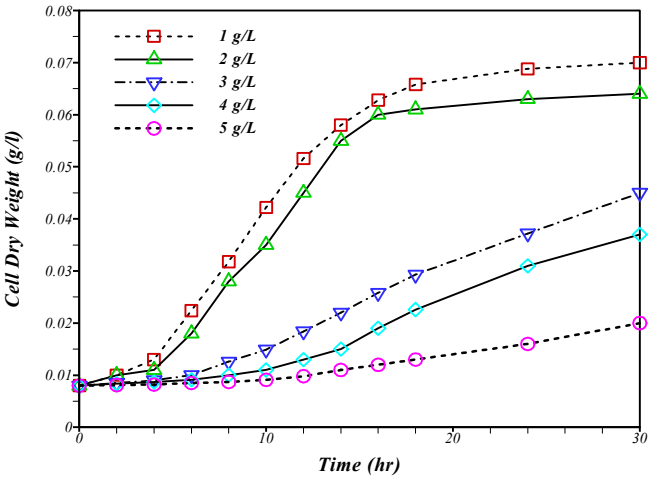

Fig. 4: Cell dry weight profile of *P. putida*

(1 to 2g/l), the removal was observed with a short lag phase. The exponential phase was completed in less than 12h for 1 and 2g/l, and then it was shifted to stationary phase.

To characterize the growth curves, logistic equation was used, which has additional benefit in predicting the entire growth curve pattern. Growth curve includes lag phase, exponential growth and stationary phase. Theoretically, cell growth rate is expressed as follows [21, 24]:

$$\frac{dX}{dt} = \mu X \quad (1)$$

where X is the cell dry weight (g/l) and μ is the specific growth rate (h^{-1}). In the logistic model, the specific growth rate is presented as follows [21, 24]:

$$\mu = \mu_m \left(1 - \frac{X}{X_m}\right) \quad (2)$$

where μ_m is the maximum specific growth rate (h^{-1}) and X_{max} is the maximum cell dry weight (g/l). Substituting equation (2) into equation (1) and performing integration, the following equation for the cell concentration was obtained [15, 21, 24]:

$$X = \frac{X_0 \exp(\mu_m t)}{1 - \left(\frac{X_0}{X_m}\right)(1 - \exp(\mu_m t))} \quad (3)$$

The above equation predicts and determines the cell growth in batch experiments. Figure 5 represents the rate of cell growth (*dX/dt*) *vs* biomass concentration where the experimental data fitted well with R^2 of 0.91. As the acetone concentration increased, the slope of the lines and the value for μ gradually increased

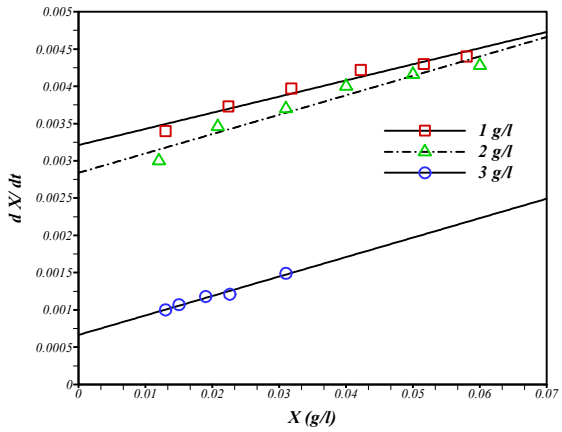

Fig. 5: The rate of cell growth (*dx/dt*) *vs* biomass concentration

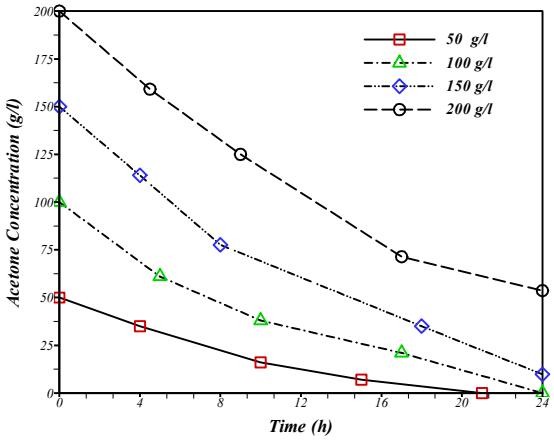

Fig. 6: Acetone concentration in the substrate tank in continuous mode

Table 1: Kinetic parameters for *P. putida* in batch experiments

Acetone Concentration	X_0	X_m	μ	R^2	X_0/X_m
1 g/l	0.008	0.07	0.0224	0.93	0.114
2 g/l	0.008	0.068	0.0255	0.91	0.117
3 g/l	0.008	0.037	0.0261	0.98	0.216

(Table 1). The linearly fitted data represent exponential growth phase for *P. putida* that follows first order rate model.

Table 1 summarizes the kinetic parameters defined in equation (3). The R^2 values for all acetone concentrations of 1, 2 and 4g/l were from 0.93, 0.91 and 0.98, respectively.

Figure 6 depicts the rapid decrease of inlet acetone concentration with respect to operation time. The substrate tank temperature was fixed at 25°C and the effect of high acetone concentrations of 50 to 200g/l was investigated. The decreasing trends of acetone in a period of 24h were about the same. For acetone concentrations of 50 and 100g/l, the acetone was fully utilized. It was definitely reported in literature that *P. putida* has the potential to oxidize aliphatic and aromatic compounds [4, 15].

Figure 7 shows the acetone removal efficiency with respect to operation time for various acetone concentrations (50 to 200g/l). The removal efficiency for 50, 100, 150 and 200g/l were 80.5, 70.5, 63 and 42 percent, respectively. Maximum removal efficiency was 80.5 percent for acetone concentration of 50g/l. As the concentration of acetone increased to 200g/l, the removal efficiency was decreased to 42 percent. In the next stage of biofiltration, at constant acetone concentration (50g/l), the effect of temperature

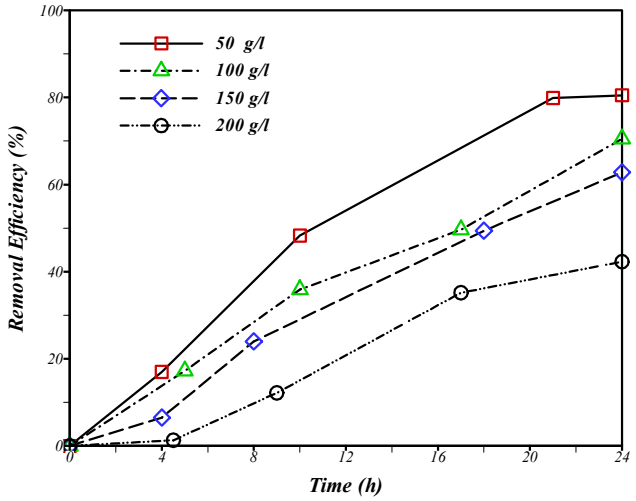

Fig. 7: Removal efficiency of acetone for various acetone concentrations in a continuous biofilter

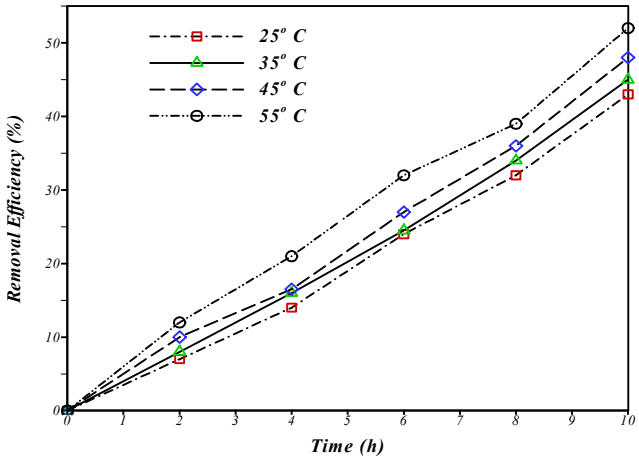

Fig. 8: Removal efficiency of acetone in a biofilter at various temperatures

(25 to 55°C) was investigated. The rate of vaporization of acetone at the elevated temperature (55°C) was higher than low temperature (25°C). While the temperature of the substrate tank was set at 55°C, acetone was completely vaporized before 10h of operation. The data for acetone removal are shown in Figure 8. The inlet gas temperature did not influence the removal efficiency, but the experimental run time for high temperature was shortened. As shown in this graph, the removal efficiency at temperatures of 25, 35, 45 and 55°C were 43, 45, 48 and 52 percent, respectively. Increasing the air stream temperature (25 to 45°C) while purging the air through the substrate tank, resulted in 9 percent increase in removal efficiency.

CONCLUSION

To demonstrate the biodegradation of organic pollutant using *P. putida*, batch experiments were accomplished. The maximum removal efficiency of 83 percent was achieved with 1g/l acetone. Batch experimental data were fitted with logistic model and kinetic parameters were obtained. In the continuous experiment, column biofilter packed with walnut shell as filter media was used. VOC was successfully removed from the contaminated air using *P. putida.* It was ensured that the filter media held the moisture with recycling the medium at constant flow rate, as the microorganisms were easily attached on the rough and natural media surface. High removal efficiency of 80.5 percent was achieved in

the biofilter with 50g/l acetone. While the acetone concentration in the feed tank increased to 200g/l, the removal efficiency dropped to 42 percent. It was observed that at fixed acetone concentration, gas temperature did not influence the biofilter removal efficiency.

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