Continuous bioremediation of phenol polluted air in an external loop airlift bioreactor with packed bed

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ABSTRACT. An External Loop Airlift Bioreactor with a small amount (99% porosity) of a stainless steel mesh packing inserted in the riser section was used for bioremediation of a phenol polluted air stream. The packing enhanced VOC and oxygen mass transfer rates and provided a large surface area for cell immobilization. Using a pure strain of *Pseudomonas putida*, batch and continuous runs at three different dilution rates were completed in this bioreactor with phenol form the polluted air as the only source of growth substrate. Essentially 100 % phenol removal was achieved at a phenol loading rate of 22160 mg/h.m^3, superior to any previously reported biodegradation rates of VOC polluted air. This bioreactor seems to be a novel bioreactor with a high potential to continuously bioremediate VOCs at high loading rates.

1 INTRODUCTION

For the past three decades, circulating loop airlift bioreactors have achieved increasing attention for treatment of contaminated air effluents (Wen *et al*., 2005; Lo and Hwang, 2004; Edwards and Nirmalakhandan, 1999). The External Loop Airlift Bioreactor (ELAB) has a simple design, without any moving parts, and it provides sufficient mixing for slow microbial reactions. In the ELAB, a specific volatile organic chemical (VOC) may be completely degraded by a microorganism at normal temperature and pressure without producing a second polluted byproduct. We have reported the use of the ELAB for bioremediation of hydrophilic (Ritchie and Hill, 1995; Wei *et al*., 1999) and hydrophobic (Harding *et al*., 2003) air pollutants. Ritchie and Hill (1995) have reported achievement of bioremediation of phenol polluted air effluent in the ELAB with a maximum phenol loading rate of 16200 mg/h.m^3. In this work, we report on a novel improvement to the ELAB, the incorporation of a packed bed in the riser section. A biofilm was fixed to the packing and greatly enhanced the biodegradation rate of phenol polluted air.

2 MATERIALS AND METHODS

2.1 Microorganism and Media

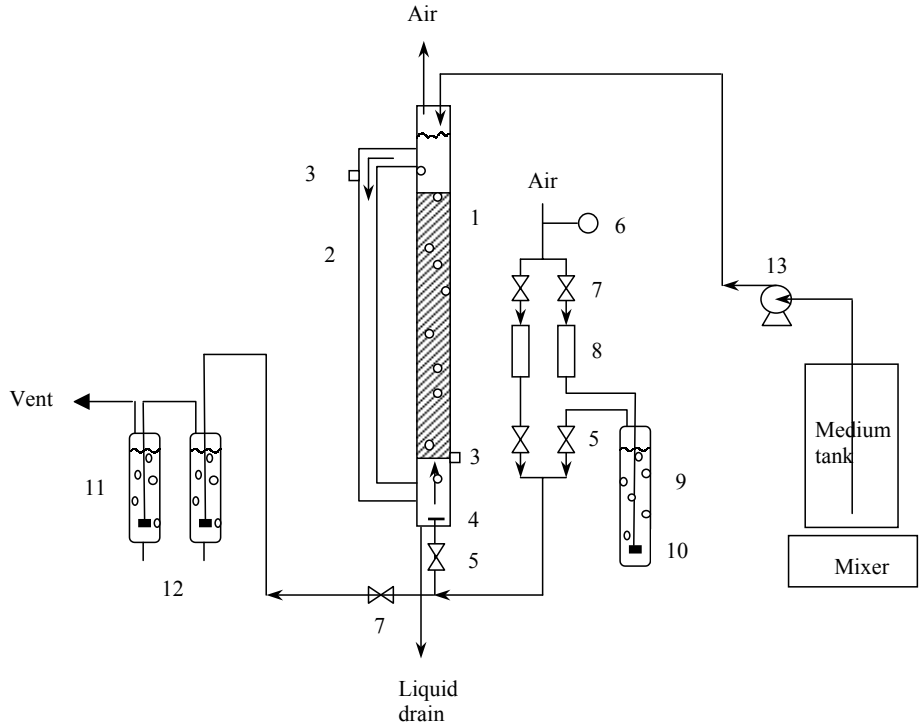
Pseudomonas putida (ATCC 17484) was used for all microbial growth experiments. It was maintained on nutrient broth agar and stored at 4 °C. The growth media consisted of (mg in 1 L reverse osmosis water; analytical reagent grade chemicals, BDH, Toronto): K_2HPO_4, 850; KH_2PO_4, 840; $(NH_4)_2SO_4$, 474; NaCl, 60; $CaCl_2$, 60; $MgSO_4$, 60; $Fe(NH_4)SO_4$, 20; and 1 ml of trace mineral solution. The trace mineral solution consisted of (mg in 1 L reverse osmosis water): $ZnSO_4.7H_2O$, 200; $MnCl_2$, 60; H_3BO_3, 300; $CoCl_2$, 400; $CuCl_2$, 20; $NiCl_2$, 40; Na_2MoO_4, 60. The pH of this solution was 6.97.

2.2 Bioremediation procedure

The same ELAB that was used in earlier work for oxygen and VOC mass transfer studies (Nikakhtari and Hill 2005a, b) was used in this study. A schematic diagram of the ELAB with the packed bed is shown in Figure 1. Riser section diameter, liquid height above sparger and riser to downcomer cross-sectional area ratio were 89 mm, 1.42 m and 3.57, respectively. The sparger included six, 1.6 mm diameter orifices. The height and porosity of the packing were 1.2 m and 99.0%, respectively.

Air was introduced to a gasifier containing 1 liter of a saturated phenol solution. The concentration of phenol in the gasifier effluent stream was measured by introducing the gas stream to a series of two bubblers. As long as the second bubbler showed zero concentration of phenol, the accumulated concentration in the first bubbler can be used to determine the phenol concentration in the air phase. Using this method, the phenol concentration in the inlet gas stream was found to remain at the saturated concentration at room temperature and pressure (0.8 mg/l, at 23 ^oC and 0.932×10^5 Pa) over all the air flow rates used in this study. The outlet air from the gasifier was introduced to the ELAB through the sparger. For all bioremediation experiments, the air flow rate was maintained by a calibrated rotameter at a constant value of 9.23×10^{-5} m^3/s which provided a gas superficial velocity of 0.0148 m/s in the riser section of the ELAB. The size of the gasifier limited the maximum air flow rate that could be used in this study. Fresh sterilized medium was pumped into the ELAB at the top and liquid was drained out of the bottom of the ELAB riser section.

One batch run and three continuous runs at different dilution rates (0.05, 0.20, and 0.50) were performed. Then the conditions used for the lowest dilution rate were repeated for an extended duration to test the stability of the bioreactor.



1- Riser section with packing
2- Downcomer section
3- Sampling port
4- Gas sparger
5- Two -way valve
6- Pressure gage
7- Adjusting valve
8- Flow meter
9- Gasifier
10- Sparger
11- Bubbler
12- Sampling valve
13- Adjustable pump

Figure 1. Schematic of the external loop airlift bioreactor.

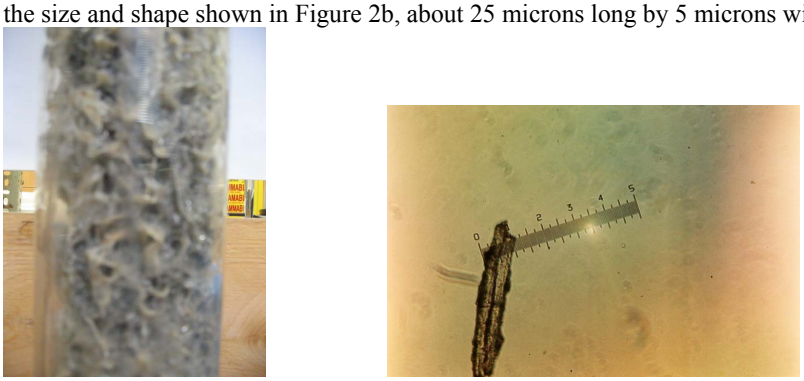
2.3 Analysis

A syringe was used to take samples from one of two ports in the riser (10 cm above downcomer inlet or 20 cm below downcomer outlet). Biomass concentrations were measured at 620 nm wavelength using a spectrophotometer (model Mandel 1240, Shimadzu, Kyoto, Japan). Optical density was converted to cell dry weight using a previously prepared calibration curve. For measurement of phenol, samples were filtered and analyzed immediately using the same spectrophotometer at 247 nm. Then absorbance was converted to phenol concentration using a prepared calibration curve.

3 RESULTS AND DISCUSSION

All phenol is absorbed in the water phase at all operating conditions. The outlet air from the ELAB does not have any phenol due to complete absorption of this hydrophilic chemical.

The developed biofilm on the stainless steel packing and a micro-photograph of detached biofilm are shown in Figure 2. The released biofilm particles were generally of the size and shape shown in Figure 2b, about 25 microns long by 5 microns wide.



(a) (b)

Figure 2. (a) Developed biofilm on the stainless steel packing, (b) Micro-photograph of detached biofilm.

Variation of phenol and free biomass concentrations during a fed-batch run are shown in Figure 3. These profiles are similar to those reported by Ritchie and Hill (1995) without a packed bed, except they used lower air superficial velocities (maximum of 0.0118 m/s) and achieved slower biodegradation rates. They achieved a maximum phenol loading rate of 16200 mg/h.m^3. In Figure 3, the experiment phenol loading rate was 22160 mg/h.m^3 (30% improvement in bioremediation rate). In their fed-batch experiments, the phenol concentration reached a negligible value after 14 hours of operation but in the present experimental work negligible phenol concentrations were achieved after only 6 hours in spite of the higher loading rate. This is believed to be due to the contribution of the biofilm to phenol capture and biodegradation.

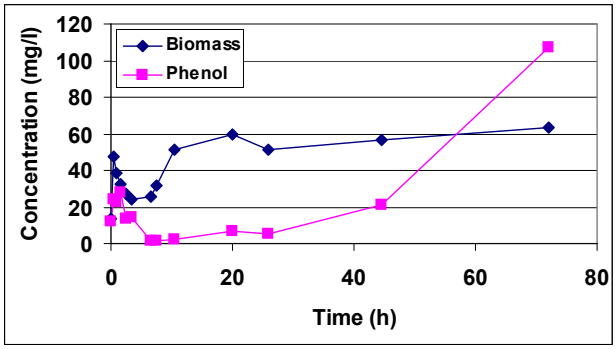


Figure 3. Phenol degradation during a fed-batch run.

The suspended biomass concentration shows an increase right after starting the air flow, which is because some of the biofilm is released from the steel mesh when the turbulent, air-liquid two phase fluid flow commences. The suspended biomass concentration shows some fluctuation with time, but appears to remain fairly constant at 60 mg/L. Ritchie and Hill (1995) reported a steadily increasing suspended biomass concentration, however in this study it is believed the bulk of the phenol consumption

goes into maintaining and increasing the biofilm which was not present in the work of Ritchie and Hill. After about 40 hours, the medium ran out of nutrients (at that time a total of 886 mg/L of phenol had been metabolized), and the phenol concentration started to increase. This demonstrates that continuous feed of nutrients is necessary to operate this bioreactor over extended periods of time, in spite of the negligible build up of suspended biomass.

To prove this, three continuous runs at different dilution rates (0.05, 0.20, and 0.50 h^{-1}) were done. The liquid flow rates were 0.6, 2.4, and 6 l/h resulting in residence times of 20, 5, and 2 h, respectively. Experiments were continued at each flowrate until steady concentrations were achieved, which resulted in durations over four residence times in each case. Figure 4 shows results of one of these continuous experiments (0.20 h^{-1} dilution rate). Again, right after start-up of the air, an increase in suspended biomass concentration was observed due to biomass being released from the biofilm. After that, because suspended biomass loss in the effluent is higher than its production rate due to growth and re-entrapment in the biofilm, its concentration falls rapidly. Steady state is reached in 40, 5, and 2 h for dilution rates of 0.05, 0.20 and 0.50 h^{-1}, respectively. Phenol concentrations increase during the early transient phase, then fall to low concentrations (below measurement accuracy) prior to achieving the steady state conditions. The maximum transient phenol concentrations reached were 14.9, 13.6, and 5.4 mg/l for dilution rates of 0.05, 0.20, and 0.50 h^{-1}, respectively. It is notable that the highest dilution rate used in this study, 0.50 h^{-1} is above the maximum growth rate of the cells, and yet a steady state condition is still achieved due to the growth activity of the fixed biomass.

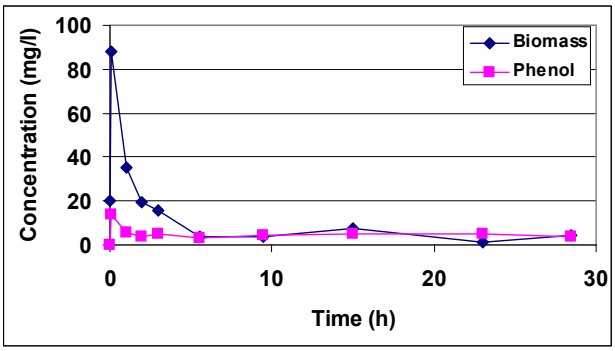


Figure 4. Continuous run at a dilution rate of 0.20 h^{-1} (liquid flow rate of 2.4 l/h).

The last experiment was a replication of the 0.05 h^{-1} dilution rate for an extended period of time to show stability of the steady state condition in the column. This final run was continued for 17 residence times, a total of 340 h, and the phenol concentration stayed below 10 mg/L. In spite of negligible amount of phenol in the water phase, which remains constant over a long period of time, no measurable amounts of phenol occur in the effluent air phase, and the polluted air problem has been completely removed. It seems that this novel packed bed, ELAB is capable of long term steady state operation with biofilm removal due to sporadic detachment caused by the turbulent bubble flow through the packing and then continuous removal of detached biofilm in the bioreactor effluent.

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