Naphthalene biodegradation kinetics in an aerobic slurry-phase bioreactor


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Abstract

The research was focused on the slurry-phase biodegradation of naphthalene in soil. Among ex situ techniques, the slurry phase offers the advantage of increased availability of contaminants to bacteria. From naphthalene contaminated soil, a Pseudomonas putida M8 strain capable to degrade naphthalene was selected.

Experiments were performed in a stirred and oxygenated reactor. In this study, the influence of air flow rate and agitation rate on volatilisation and biodegradation of naphthalene was investigated. The hydrocarbon disappearance, the carbon dioxide production, and the ratio of total heterotrophic and naphthalene-degrading bacteria was monitored. The results obtained confirm that the selected bioremediation technology is successful in the treatment of contaminated soils.

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1. Introduction

Bioremediation of contaminated soils offers a number of advantages over conventional treatments on the basis of its environmental friendliness and low costs. The interest in this technology has increased over the last few years (USEPA, 2001).

Many factors influence the feasibility and effectiveness of bioremediation, among others the presence of suitable microorganisms, the availability of nutrients, temperature, and pH (Alexander, 1999). Consideration of these factors is critical for the successful implementation of bioremediation systems.

Bioremediation applications fall into two broad categories: in situ and ex situ techniques. In this research project, ex situ techniques are selected, being faster, easier to control, and more useful to treat a wider range of contaminants and soil types than in situ techniques (Hinchee and Offenbittel, 1991; King et al., 1992; Park et al., 2001). Among ex situ techniques, slurry phase is chosen, where contaminated soil is combined with water and other additives in a bioreactor and then mixed. Nutrients and oxygen are added, and conditions in the bioreactor are controlled to create the optimum environment for microorganisms to degrade the contaminants. This technique offers two advantages. First, these experimental conditions maximise the contact between the solid and the aqueous phase, thus enhancing the mass transfer and, as a consequence, the biodegradation rate. In addition, slurry-phase degradation experiments give results that can be promptly transferred to a full-scale process (Alleman and Leeson, 1999; Freeman and Harris, 1995).

Slurry-phase biodegradation experiments were performed in a bioreactor comprised of a 1 L vessel with a stirring apparatus and air sparger. To estimate the biodegradation efficiency, we monitored the disappearance of naphthalene, the carbon dioxide evolution, and the total heterotrophic and hydrocarbon-degrading bacteria.

From naphthalene contaminated soil, a Pseudomonas putida M8 strain capable to degrade naphthalene was selected. Process parameters as naphthalene concentration, soil/water ratio, and inoculum concentration have been...
optimised in preliminary experiments in slurry aerobic microcosms (Bestetti et al., 2003).

In this paper, we present slurry-phase bioreactor experiments where the air flow and agitation rates were modified to investigate their influence on naphthalene volatilisation and biodegradation rate.

2. Materials and methods

2.1. Soil samples

Soil for the experiments was collected from a non-contaminated area. Soil organic carbon content was 2.74%, and water content was 6.83%. Soil was sieved; the fraction < 2 mm was sterilised and used in the slurry-phase experiments. A previous study showed that the indigenous microbial population did not have any effect on the rate of naphthalene degradation in the presence of the \textit{P. putida} M8 strain (Bestetti et al., 2003). This fraction was added with the required amount of naphthalene dissolved in dichloromethane to reach the concentration of 13.5 mg/g dry soil, the mixture was homogenised and dried.

2.2. Preparation of inoculum

The \textit{P. putida} M8 strain was maintained in M9 mineral medium (Maniatis et al., 1982) supplied with naphthalene as the only carbon and energy source at 30°C. Growth was performed in M9 mineral medium either in liquid or solid with 15 g/L agar. M8 liquid cultures were prepared in 100 mL of M9 mineral medium with 500 ppm of naphthalene, added in crystalline form. The cultures were incubated in a rotary shaker overnight at 30°C. The cells were removed by centrifugation, washed and resuspended in M9 mineral medium to obtain the desired initial optical density at 540 nm (OD\textsubscript{540}).

2.3. Bioreactor design

The volume of the reactor was 1000 mL. The reactor temperature was maintained at 30°C with a thermostat jacket. The agitation rate was controlled by a stirring velocity regulator. Oxygenation was provided by injection, through a porous diffuser, of synthetic air, i.e., an oxygen-nitrogen mixture (20% and 80% in volume, respectively). The outgoing flow passed through an empty 250 mL Drechsel bottle to sample the reactor atmosphere for carbon dioxide analysis.

2.4. Kinetic experiments

The reactor was added with 200 g of soil contaminated with naphthalene and 544 mL of M9 mineral medium to obtain a slurry phase with a dry soil/water ratio of 1:3. The slurry phase was added with the inoculum at OD\textsubscript{540}=1.

At the programmed kinetic times, four slurry samples of about 3 mL were collected from the reactor and centrifuged, one for microbiological analyses and three for naphthalene quantification. Carbon dioxide concentration flowing through the Drechsel bottle was determined at regular intervals.

Experiments were performed with two different air flow rates (0.1 and 0.05 L/min) and four different agitation rates (130, 200, 350, and 500 rpm). For each tested combination of air flow rate and agitation rate, one additional slurry-phase experiment was performed as control to evaluate the abiotic loss of naphthalene.

2.5. Analytical procedures

2.5.1. Evaluation of total heterotrophic and naphthalene-degrading bacteria

The centrifuged slurry sample was serially diluted in M9 mineral medium. The 0.1 mL dilutions were transferred on agar plates of suitable medium. Plates were incubated at 30°C for 48 h. The colony forming units per gram of dry soil (CFU/g\textsubscript{dw}) were determined. The media used were LD medium for total heterotrophic bacteria and M9 mineral medium with naphthalene as the only carbon and energy source for the hydrocarbon-degrading bacteria.

2.5.2. Naphthalene quantification

For the determination of residual naphthalene, the centrifuged slurry sample was added with 30 mL dichloromethane (Sigma-Aldrich, 99.6%). Deuterated naphthalene (Sigma-Aldrich, 98.9%), used as internal standard, was added in appropriate amounts in the range 0.1–10 mg. The bottle was sealed with a Teflon stopper and held for 10 min in an ultrasonic bath (Branson MT2210) at 47 kHz frequency; the extract was filtered on anhydrous sodium sulphate (CODEX Carlo Erba, 97%), dried, dissolved in hexane, and analysed.

The analyses were performed with an HP 5890 gas chromatograph (CP-Sil 8 CB column, Chrompack) coupled to an HP 5970 mass selective detector. The temperature program was 1 min at 60°C, then 20 °C min\textsuperscript{-1} to 280°C, and 4 min at 280°C.

2.5.3. Carbon dioxide quantification

To determine the carbon dioxide concentration in the reactor, the Drechsel was purged with 50 mL/min air flow, controlled by a Flow Meter/Controller (MKS, mod. 2259C) connected to a control unit (MKS, Multi Gas Controller mod. 647B). The effluent was isothermally analysed with an HP 5890 gas chromatograph series II (Porapak Q and Molecular sieve columns) coupled to a TCD detector at a temperature of 200°C.
3. Results and discussion

Slurry-phase biodegradation experiments were performed at different air flows (0.1 and 0.05 L/min) and agitation rates (130, 200, 350, and 500 rpm) to investigate the influence of these parameters on naphthalene volatilisation and biodegradation.

Fig. 1 shows, as an example, the decrease in time of naphthalene in slurry-phase bioreactor treatments with 0.1 L/min air flow rate and 350 rpm agitation rate in inoculated as well as noninoculated abiotic systems. Error bars are also reported. Fig. 2 shows for the inoculated system in the same conditions the carbon dioxide production rate.

We observed that for each set of conditions tested, naphthalene disappearance in the abiotic system was slower than in the inoculated system. In all experiments, both inoculated and abiotic, the decrease of naphthalene followed a pseudo-first-order kinetics.

The time for which the maximum disappearance rate of naphthalene was observed was not coincident with the time of maximum CO2 production rate. This delay was probably due to the accumulation of intermediate metabolites before complete mineralisation.

During all biotic experiments, the number of total heterotrophic and naphthalene degrading-bacteria was almost coincident and remained constant with values around $10^7$ CFU/g dw.

In the bioreactor, naphthalene disappearance was due to both biotic (complete mineralisation, production of biomass, and production of metabolites) and abiotic (volatilisation) processes (Fig. 3). In inoculated experiments, both biotic and abiotic processes were involved, while, in abiotic systems, only volatilisation of naphthalene took place.

On the basis of experimental evidence, we hypothesised that both biodegradation and volatilisation followed a pseudo-first-order kinetics, characterised by the kinetic constants $k_{bio}$ and $k_{vap}$, respectively.

Assuming first-order behaviour also for the transformation of metabolites to CO2+H2O and to biomass, the model can be expressed, in differential form, as follows (Moore and Pearson, 1981):

$$\frac{dA_1}{dt} = -k_{bio}A_1 - k_{vap}A_1 = -(k_{bio} + k_{vap})A_1 = -k_{tot}A_1$$

$$\frac{dA_2}{dt} = -k_{23}A_2 - k_{24}A_2 + k_{bio}A_1$$

$$\frac{dA_3}{dt} = \frac{dCO_2}{dt} = k_{23}A_2$$

Values for $k_{tot}$ and $k_{vap}$ were determined by least-squares regression of data from biotic and abiotic experiments, respectively. The constant $k_{bio}$ was obtained as the difference between $k_{tot}$ and $k_{vap}$. The three kinetic constants, together with the determination coefficient from least squares regression, $R^2$, and the standard errors are reported in Table 1.

CO2 production rate values (mmol 10$^{-1}$ h$^{-1}$, the factor 10 coming from the stoichiometry of naphthalene transformation to CO2) were fitted with following expression:

$$\frac{dCO_2}{dt} = \frac{A_0k_{23}k_{bio}}{(k_{23} + k_{24} - k_{bio} - k_{vap})} \{\exp[-(k_{bio} + k_{vap})t] - \exp[-(k_{23} + k_{24})t]\}$$

The CO2 data fitting gave appreciable results only for experiments with 0.1 L min$^{-1}$ air flow rate. Fig. 2 shows, as an example, the good fitting of CO2 production rate data for 0.1 L min$^{-1}$/350 rpm experiments.

Mineralisation ratio $R_{min}$, i.e., the ratio of CO2 produced to naphthalene biodegraded, was calculated by:

$$R_{min} = 100 \times \frac{k_{23}(k_{bio} + k_{vap})}{(k_{23} + k_{24} - k_{bio} - k_{vap})}$$
The values calculated for experiments with 0.1 L min\(^{-1}\) air flow rate and agitation rates of 200, 350, and 500 rpm were 35, 57, and 34, respectively.

From data reported in Table 1, it is possible to make some considerations about the influence of process parameters on \(k_{\text{bio}}\), \(k_{\text{tot}}\) and \(k_{\text{bio}}/k_{\text{tot}}\).

Regarding \(k_{\text{vap}}\), it is evident that, at constant air flow rate, its values are positively correlated with the agitation rate, while the two values measured at fixed agitation rate are not significantly different. Thus, we conclude that \(k_{\text{vap}}\) is only dependent on the agitation rate.

Regarding \(k_{\text{bio}}\), its dependence on the process parameters is more complex. Obviously, the oxygen concentration is directly dependent on the air flow rate. Moreover, the influence of agitation rate is double. On one hand, stirring increases the contact between the reagents (naphthalene, oxygen, and biomass), thus enhancing the mass transfer and, as a consequence, the biodegradation rate, \(k_{\text{bio}}\). On the other hand, agitation increases the contact surface between the slurry phase and the gas phase in the bioreactor, thus enhancing the loss by volatilisation of oxygen and naphthalene and decreasing the biodegradation rate. Hence, due to these effects, \(k_{\text{bio}}\) shows a maximum for tested intermediate values of agitation rate: 350 rpm for 0.1 L/min air flow rate experiments and 200 rpm for 0.05 L/min air flow rate experiments.

Although the CO\(_2\) data fitting gave appreciable results only for experiments with 0.1 L min\(^{-1}\) air flow rate, mineralisation ratio values confirmed that 350 rpm and 0.1 L min\(^{-1}\) was the best condition tested.

### Table 1
Kinetik constants for slurry-phase biodegradation.

<table>
<thead>
<tr>
<th>Air flow rate (L/min)</th>
<th>Agitation rate (rpm)</th>
<th>(k_{\text{vap}}) (h(^{-1}))</th>
<th>(R^2)</th>
<th>(k_{\text{tot}}) (h(^{-1}))</th>
<th>(R^2)</th>
<th>(k_{\text{bio}}) (h(^{-1}))</th>
<th>(k_{\text{bio}}/k_{\text{tot}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>130</td>
<td>0.018±0.003</td>
<td>0.929</td>
<td>0.165±0.002</td>
<td>0.999</td>
<td>0.147±0.005</td>
<td>0.89±0.04</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.051±0.007</td>
<td>0.983</td>
<td>0.29±0.03</td>
<td>0.964</td>
<td>0.239±0.007</td>
<td>0.82±0.03</td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>0.063±0.003</td>
<td>0.950</td>
<td>0.11±0.02</td>
<td>0.994</td>
<td>0.05±0.02</td>
<td>0.5±0.3</td>
</tr>
<tr>
<td>0.1</td>
<td>200</td>
<td>0.024±0.006</td>
<td>0.799</td>
<td>0.14±0.01</td>
<td>0.984</td>
<td>0.12±0.01</td>
<td>0.86±0.07</td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>0.06±0.01</td>
<td>0.915</td>
<td>0.36±0.02</td>
<td>0.994</td>
<td>0.30±0.02</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.07±0.01</td>
<td>0.901</td>
<td>0.26±0.02</td>
<td>0.952</td>
<td>0.19±0.03</td>
<td>0.7±0.2</td>
</tr>
</tbody>
</table>

### 4. Conclusions

Air flow rate and agitation rate are important factors affecting the efficiency of biodegradation in a slurry-phase bioreactor and show, for fixed air flow rate, an optimum intermediate value of agitation rate to maximise biodegradation and minimise volatilisation.

Laboratory scale experiments are very useful in determining optimal operating conditions in the slurry-phase bioreactor. The results show that in the adopted experimental condition, high biodegradation rate and mineralisation ratio values are observed.

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References


