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Improved mass transfer and biodegradation rates of naphthalene particles using a novel bead mill bioreactor

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Abstract: One of the main challenges in the treatment of polycyclic aromatic hydrocarbons (PAHs) in controlled bioreactors is the hydrophobicity and low solubility of these compounds in the aqueous phase, resulting in appreciable mass transfer limitations within the bioreactor. To address this challenge, we have developed a modified roller bioreactor (Bead Mill Bioreactor) in which inert particles are used to improve mass transfer from the solid phase to the aqueous phase. Experimental results with naphthalene as a model PAH and *Pseudomonas putida* as a candidate bacterium indicate that both the mass transfer rate from the solid phase to liquid phase and the biodegradation rate in the Bead Mill Bioreactor (BMB) were significantly higher than those in a conventional roller bioreactor (20-fold and 5.5-fold, respectively). The enhancement of mass transfer was dependent on the type, size and volumetric loading of the inert particles, as well as concentration of particulate naphthalene. The highest mass transfer coefficient ($k_L a = 2.1\,h^{-1}$) was achieved with 3 mm glass beads at a volumetric loading of 50% (particle volume/working volume) with 10 000 mg dm^{-3} particulate naphthalene. The maximum biodegradation rate of naphthalene attained in the bead mill bioreactor (59.2 mg dm^{-3} h^{-1} based on the working volume and 118.4 mg dm^{-3} h^{-1} based on the liquid volume) surpasses most other rates published in the literature and is equivalent to values reported for more complex bioreaction systems. The bead mill bioreactor developed in the present work not only enjoys a simple design but shows excellent performance for treatment of PAHs suspended in an aqueous phase. This fundamental information will be of significant value for future studies involving soil-bound PAHs.

Keywords: polycyclic aromatic hydrocarbons; mass transfer; biodegradation; *Pseudomonas putida*; bead mill bioreactor

INTRODUCTION

Industrial activities and agricultural developments release a vast amount of hazardous compounds into the environment. Polycyclic aromatic hydrocarbons (PAHs) of natural and anthropogenic origins form a major portion of these contaminants.[1] PAHs constitute a diverse class of organic compounds consisting of two or more benzene rings fused in linear, angular or cluster oreientations.[2,3] The structural characteristics of PAHs such as number of rings, angularity, as well as their low solubility and high hydrophobicity make them persistent in the environment.[4] PAHs are widely distributed in the environment, particularly in industrial sites associated with petroleum and gas processing.[5,6] In western Canada, PAHs and recalcitrant naphthenic acids, which occur with a diverse range of hydrocarbons in oil sand deposits, pose a serious threat to the environment. Polycyclic aromatic hydrocarbons are amenable to biodegradation by bacterial and fungal species[1,7] and a variety of inexpensive bioremediation technologies such as landfarming and composting have been used for treatment of contaminated sites.[8]

Biotechnologies for treatment of PAH-contaminated sites can be classified as *in-situ* and *ex-situ* processes. *In-situ* bioremediation is a very slow process and cannot be controlled effectively. Treatment of the contaminated soil and liquid streams in controlled bioreactors, on the other hand, converts these hazardous contaminants into less harmful compounds in an efficient manner. One of the main barriers to successful *ex-situ* bioremediation of PAHs and recalcitrant naphthenic acids is the hydrophobicity and low solubility of these compounds in the aqueous phase, resulting in significant mass transfer limitations within the bioreactor.[9–11] Stirred tank bioreactors

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offer an advantage with respect to mass transfer from the solid phase to the liquid phase, since the dissolution rate can be increased by applying a proper agitation strategy. However, inherent to the design of stirred tank bioreactors, solid, hydrophobic particles cling to the agitation system, baffles and walls. This, together with lifting and separation of particles from the liquid phase by sparged air, required for the metabolism of the microbial cells, prevents the efficient biodegradation of solid PAHs.[12] Application of a conventional roller bioreactor circumvents these problems to some extent.[12–16]

Biodegradation of naphthalene as a model PAH with *Pseudomonas putida* has been studied in our previous work, both in the stirred tank and roller bioreactors.[12] Although efficient solid to liquid mass transfer was achieved in the stirred tank bioreactor (volumetric mass transfer coefficient: $5\,h^{-1}$) the extensive stripping loss of naphthalene, due to splashing and aeration, made the use of a stirred tank bioreactor for biodegradation of naphthalene impractical. The stripping of naphthalene was overcome by using the roller bioreactor but significant mass transfer limitation (volumetric mass transfer coefficient: $0.055\,h^{-1}$) prevented the efficient biodegradation of naphthalene.[12]

In this study we report on development of a novel Bead Mill Bioreactor (BMB) in which inert particles are used as a means to create efficient mixing and improve the extent of mass transfer and biodegradation rates. Quantitative data obtained in the BMB indicate a significant improvement of naphthalene mass transfer from the solid phase to the liquid phase. The rate of increase of naphthalene bioremediation is also presented.

MATERIALS AND METHODS

Microorganism and medium

The microorganism used in this study was *Pseudomonas putida* (ATCC 17 484). This aerobic bacterium is chemoheterotrophic, capable of growth over a range of temperatures in a neutral pH environment.[17] The cells were stored on nutrient broth agar plates at 4 °C. The mineral nutrient medium used to support the growth of *P putida* was McKinney's modified medium, as used by Hill and Robinson.[18] The medium contained, per dm^{-3}: 420 mg KH_2PO_4, 375 mg K_2HPO_4, 237 mg $(NH_4)_2SO_4$, 30 mg NaCl, 30 mg $CaCl_2$, 30 mg $MgSO_4$, 10 mg $Fe(NH_4)_2SO_4$ and 1 cm^3 of a trace element solution. The trace element solution contained, per dm^{-3}: 300 mg H_3BO_3, 200 mg $CoCl_3$, 100 mg $ZnSO_4.7H_20$, 30 mg $MnCl_2$, 30 mg Na_2MoO_4, 20 mg $NiCl_2$, and 10 mg $CuCl_2$. The final pH of the medium was 6.8. Liquid cultures were prepared by transfer of bacterial colonies from agar plates to 125 cm^3 of sterile McKinney's modified medium containing 250 mg dm^{-3} of particulate naphthalene as growth substrate in 250 cm^3 Erlenmeyer flasks. The stock liquid cultures were maintained by subculturing on a bi-weekly basis.

Apparatus and experimental procedures

Roller bioreactors were 2.5 dm^3, narrow-mouthed glass jars (inside diameter of 12.5 cm, length of 21 cm), rotated on a Bellco Biotechnology roller apparatus (model 7622-S0003, New Jersey, USA) at 50 rpm. The bioreactors were operated with 1 dm^3 working volume (volume of the liquid in control experiments; volume of liquid plus the volume of inert particles in other cases) at 22 ± 1 °C. Teflon caps, placed over the roller jar openings, allowed sampling and intermittent injection of air into the bioreactors. The caps were equipped with filtered air vents.

In order to generate efficient mixing and improve the extent of mass transfer, inert particles (glass beads or glass Raschig rings) were added to the roller bioreactor. To assess the effect of particle loading on the extent of mass transfer various quantities of 3 mm glass beads (10, 25, 50, 75%; volume of glass beads/total working volume) were added to the roller bioreactors containing McKinney's modified medium and 1000 mg dm^{-3} of suspended naphthalene particles. The total working volume (initial volume of the medium plus the glass beads) was kept constant at 1 dm^3. The roller bioreactor was then operated at a rotational speed of 50 rpm and the concentration of dissolved naphthalene was monitored as a function of time until the naphthalene concentration reached the saturation level. Similar experiments were carried out using 500, 5000 and 10 000 mg dm^{-3} of naphthalene particles.

The effect of bead size on the extent of mass transfer was investigated using glass beads with diameters of 1, 3 and 5 mm. In each case the roller bioreactor was charged with 500 cm^3 of McKinney's modified medium, 1000 mg of naphthalene particles and 500 cm^3 of glass beads (50% loading). In one set of experiments, glass Raschig rings (id = 3 mm, od = 6 mm, L = 5mm) were used instead of glass beads. In all cases, control experiments were carried out under similar operating conditions but without adding glass beads or Raschig rings.

Biodegradation of naphthalene was carried out in the presence and absence of glass beads. The bioreactors were charged with double concentrations of nutrient medium and various quantities of 3 mm glass beads (10–75% loading). The bioreactors, together with the nutrient medium and glass beads, were sterilized at 121 °C. Measured amounts of naphthalene (500 mg) were aseptically added to the cooled glass jars through a sterile funnel. The bioreactors were inoculated with 25–30 cm^3 of a fresh, actively growing culture of *P putida*. Initial culture optical density measured at 620 nm was around 0.1–0.15, which corresponds to a biomass concentration of 53–79 mg dry weight dm^{-3}. The working volume of the bioreactor was then set to 1 dm^3 by aseptic addition of sterilized distilled water.

Samples of 10 cm^3 were taken at various time intervals by pouring the broth out of the well-shaken roller jar through the cap tubing. The sample was mixed with ethanol to dissolve all naphthalene which was then quantified by HPLC, as described below. In some experiments, duplicate samples were collected and analyzed. One biodegradation experiment was carried out using 5 mm glass beads at a loading of 50%, and a control experiment was conducted under similar conditions in the absence of glass beads.

Analytical procedures and chemicals

Slurry samples were first allowed to settle for 2 min to remove large naphthalene particles. The supernatant was then passed through a 0.2 μm nylon filter membrane and the filtrate was used to measure the concentration of dissolved naphthalene. To measure the total concentration of naphthalene (dissolved and suspended), ethanol was added to fresh slurry samples (volumetric ratio of ethanol to aqueous sample: 2/1). The function of ethanol was to completely dissolve the particulate naphthalene. After addition of ethanol, the naphthalene sample was shaken on a vortex mixer for 1 min and then filtered through a 0.2 μm nylon filter membrane to remove biomass.

A high performance liquid chromatograph (HPLC, Hewlett Packard (Mississauga, Ontario, Canada) model 1100) was employed to determine naphthalene concentrations. A 20 mm^3 volume of sample was injected into a 15 cm C18 NovaPak column (Waters, Mississauga, Ontario, Canada) at a temperature of 25 °C. The mobile phase, a 50/50% (v/v) mixture of MilliQ-water (Millipore, Nepean, Ontario, Canada) and acetonitrile, was pumped through the column at a flow rate of 2.1 $cm^3\ min^{-1}$ and an average column pressure of 147 bar. A UV detector at 254 nm was used for the detection and analysis of the samples. Using known particulate slurry concentrations up to 1000 mg dm^{-3}, this technique for measuring naphthalene concentration was found to be accurate and reproducible to within ±2%.

Naphthalene with a purity of 99.5% was purchased from Sigma-Aldrich, Oakville, Ontario, Canada. Naphthalene particles were ground and sieved to produce a uniform particle size with Sauter mean diameters of 70 μm (standard deviation: 39 μm). Glass beads were obtained from Potters Canada, Moose Jaw, Canada. Other chemicals used for analyses in these experiments included absolute ethanol and HPLC grade acetonitrile.

RESULTS AND DISCUSSION

The effect of glass bead loading on the extent of mass transfer at four different initial concentrations of particulate naphthalene (500, 1000, 5000 and 10 000 mg dm^{-3}) is shown in Fig 1. As can be seen, in all cases and regardless of initial concentration

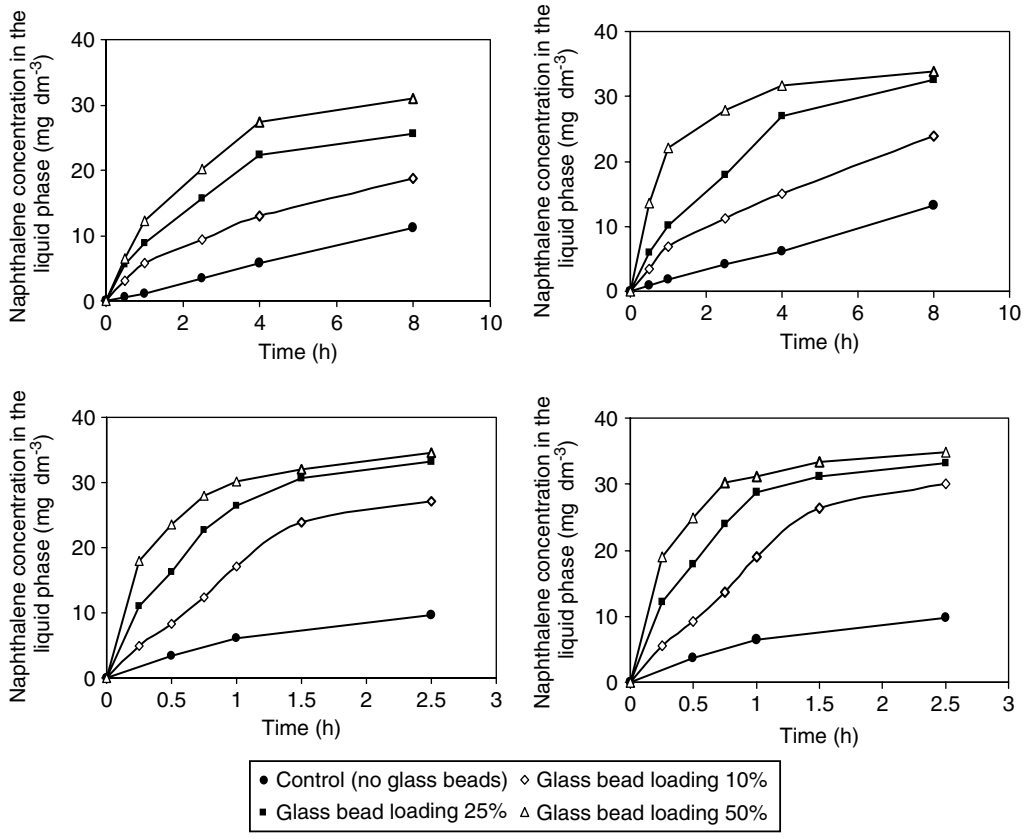

Figure 1. Dissolution of naphthalene at different loadings of glass beads and various initial concentrations of particulate naphthalene (A: 500 mg naphthalene dm^{-3}; B: 1000 mg naphthalene dm^{-3}; C: 5000 mg naphthalene dm^{-3}; D: 10 000 mg naphthalene dm^{-3}; concentrations of particulate naphthalene are based on the total working volume).

of particulate naphthalene, the addition of glass beads greatly enhanced the rate of mass transfer of naphthalene from the solid phase to the liquid phase. The dissolution rate was dependent on the loading of glass beads, and faster rates were observed when the loading of glass beads was increased from 10% to 50%. Further increase of glass bead loadings to 75% resulted in dissolution rates that were identical to those observed at 50% (data not shown).

In a batch system, the mass transfer of naphthalene from the surface of a particle through the liquid film around the particle can be described by the following differential equation:

$$V\frac{dC_L}{dt} = k_L a V(C_L^* - C_L) \quad (1)$$

where:

C_L : concentration of naphthalene in the bulk liquid ($mg\,dm^{-3}$)
C_L^* : saturated concentration of naphthalene in the liquid film adjacent to solid ($mg\,dm^{-3}$)
t : time (h)
$k_L a$: local overall mass transfer coefficient, liquid side (h^{-1})
V : liquid volume (dm^{-3})

Integration of eqn (1) yields:

$$\ln\left(\frac{C_L^* - C_L}{C_L^*}\right) = -k_L a\, t \quad (2)$$

To assess the effects of glass bead loading and initial concentration of particulate naphthalene on the extent of mass transfer, the experimental data were fitted to eqn (2) and the local mass transfer coefficients ($k_L a$) were calculated (regression coefficients in all cases were in the range 96–99%). The dependency of the volumetric local mass transfer coefficient on the concentration of particulate naphthalene and the loading of glass beads is shown in Fig 2. As can be seen, the value of the mass transfer coefficient in the presence of glass beads is significantly higher than that in the control system. Furthermore, an increase in the loading of glass beads up to 50% resulted in mass transfer enhancement. At a constant bead loading, the extent of mass transfer was enhanced as the initial concentration of particulate naphthalene was increased from 500 to 10 000 $mg\,dm^{-3}$. For instance, at a bead loading of 50% the value of $k_L a$ was around $0.3\,h^{-1}$ when the particulate naphthalene concentration was $500\,mg\,dm^{-3}$, but in the presence of 10 000 $mg\,dm^{-3}$ naphthalene a significantly higher $k_L a$ value of $2.1\,h^{-1}$ was determined. This is expected, since higher particulate concentrations increase the specific solid surface area for mass transfer to the liquid phase.

The effects of type and size of the inert particles on the dissolution of $1000\,mg\,dm^{-3}$ of naphthalene are shown in Fig 3. The application of Raschig rings at a loading of 50% improved the extent of mass transfer compared with the control. However, compared with spherical glass beads, Raschig rings produced lower mass transfer rates. The extent of mass transfer was dependent on the size of the spherical glass beads and an increase in the particle diameter from 1 mm to 5 mm, due to a more effective mixing and breakage of the naphthalene particles, improved the mass transfer rate. The volumetric mass transfer coefficient determined in the presence of Raschig rings, 1 mm, 3 mm and 5 mm glass beads were 0.3, 0.4, 0.5 and $0.7\,h^{-1}$, respectively. It appears that the Raschig rings do not provide the same crushing force and mixing turbulence that rolling glass beads were able to generate at the same loading in the BMB.

Purwaningsih *et al*[12] had earlier compared the mass transfer and biodegradation of naphthalene in conventional stirred tank and roller bioreactors. Operating the stirred tank reactor at an agitation speed of 175 rpm and naphthalene particulate concentration of $1000\,mg\,dm^{-3}$, a volumetric mass transfer coefficient ($k_L a$) of $5\,h^{-1}$ was determined. This value of $k_L a$ was

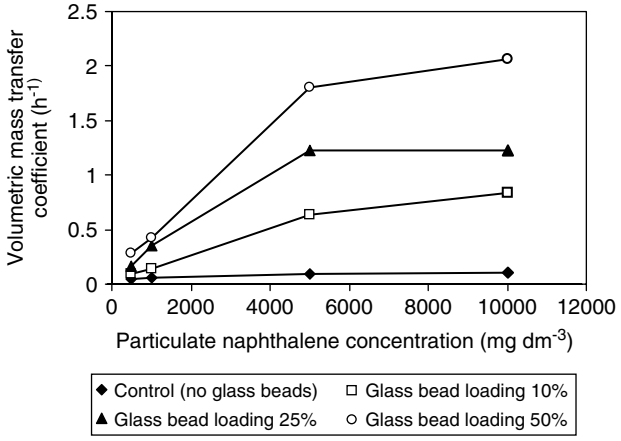

Figure 2. Dependency of volumetric mass transfer coefficient on concentration of particulate naphthalene (based on the total working volume) and loading of glass beads.

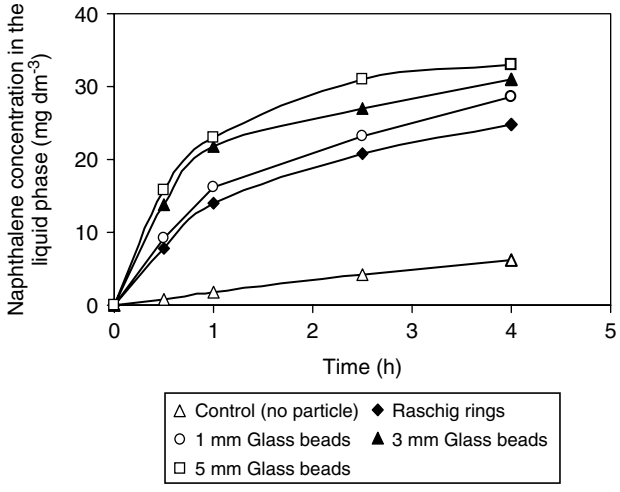

Figure 3. Dissolution of $1000\,mg\,dm^{-3}$ of particulate naphthalene in the presence of Raschig rings and glass beads of different sizes (particle loading: 50%).

two orders of magnitude higher than that achieved in the roller bioreactor, operated at a rotational speed of 50 rpm ($k_La = 0.05\,h^{-1}$). Despite a high mass transfer rate, Purwaningsih *et al* found that the use of a conventional continuous stirred tank reactor (CSTR) for biodegradation of PAHs was impractical. This was due to extensive stripping of naphthalene particles by splashing and aeration. The stripping coefficients determined for the CSTR and roller bioreactor were 0.94 and $0.017\,h^{-1}$, respectively. Mulder *et al*[11] had also studied the mass transfer of naphthalene in a stirred tank reactor. The value of k_La at impeller rotational speeds of 200 and 400 rpm was reported to be 0.08 and $0.14\,h^{-1}$, respectively. Investigating the mass transfer of naphthalene, dissolved in silicon oil and coated as a film on the surface of 3 mm polytetrafluoroethylene beads in a completely mixed reactor, Alshafie and Ghoshal[19] determined a value of 0.54 (h^{-1}) for k_La($k_L = 1.15 \times 10^{-3}\,cm\,s^{-1}$). The k_La values reported here for naphthalene in the BMB are therefore similar or greater in magnitude to those that have been reported in mixed tank reactors.

The results of biodegradation of $500\,mg\,dm^{-3}$ of particulate naphthalene in a roller bioreactor with no beads, in the presence of 3 mm glass beads at two different loadings of 25 and 50%, and with 5 mm beads at 50% loading are presented in Fig 4. Biodegradation of naphthalene in the roller bioreactor with no beads proceeded at a relatively constant rate of $10.6\,mg\,dm^{-3}\,h^{-1}$ (regression coefficient: 0.99), calculated using the total working volume of $1\,dm^3$ (glass beads plus liquid). The rate of naphthalene biodegradation in the presence of glass beads was significantly faster than that observed in the control system, with the rate being dependent on the loading of the beads. With 25% loading, complete removal of 500 mg naphthalene was achieved in 17 h, while application of beads at a loading of 50% reduced the required time for complete removal of the naphthalene to 9 h. The corresponding biodegradation rates at 25 and 50% bead loadings were $30.2\,mg\,dm^{-3}\,h^{-1}$ (regression coefficient: 0.98) and $57.6\,mg\,dm^{-3}\,h^{-1}$ (regression coefficient: 0.97), respectively. In all cases, following a short lag phase needed for establishment of a sizeable population, the linear decrease in concentration of naphthalene was observed.

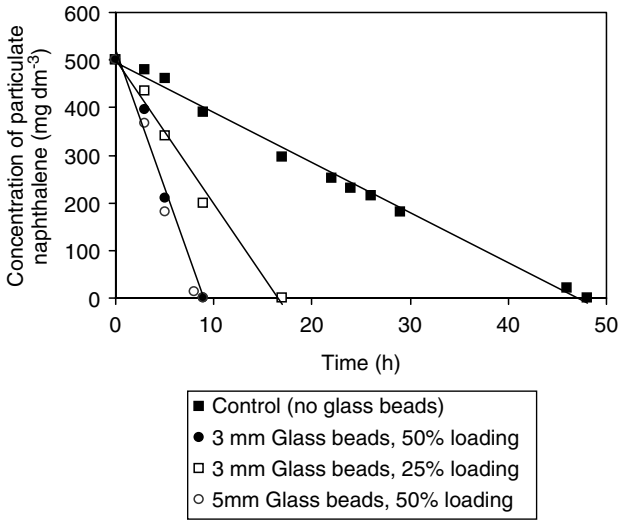

Figure 4. Biodegradation of $500\,mg\,dm^{-3}$ of particulate naphthalene in the conventional roller bioreactor (control) and in the bead mill bioreactors with 25 and 50% loadings of 3 mm glass beads, and 50% loading of 5 mm beads.

As compared with 3 mm glass beads, application of 5 mm glass beads at a loading of 50% did not offer a significant advantage and the observed biodegradation rate ($59.2\,mg\,dm^{-3}\,h^{-1}$; regression coefficient: 0.98) was only slightly higher than that obtained with 3 mm beads at the same loading (Fig 4). The similar rates observed with 3 and 5 mm beads could be explained by the fact that the measured biodegradation rate is influenced by mass transfer, as well as intrinsic naphthalene biodegradation kinetics. Although, with 5 mm beads a significantly higher mass transfer rate was achieved, the limitation imposed by the growth kinetics resulted in similar biodegradation rates. The existence of strong shear forces in the presence of 5 mm beads could be another possible explanation for a decrease in bacterial activity. However, disruption of microbial cells in bead crushers usually occurs at very large shear rates (rotational speeds as high as 2500–5000 rpm) and the mild conditions applied in our system should not severely disrupt bacterial activity.

The use of the present bead mill bioreactor not only increased the rate of mass transfer but also led to significant enhancement of the naphthalene biodegradation rate. Table 1 summarizes the values of naphthalene biodegradation rates in batch systems as reported by different researchers in recent years. The assessment and comparison of the reported biodegradation rates is rather difficult. This is due to variation in microbial species and strains, experimental conditions, as well as differences in bioreactor configurations which were employed. Nonetheless, a comparison of the available data indicates that the maximum biodegradation rate achieved in the present work ($59.2\,mg\,dm^{-3}\,h^{-1}$ based on the working volume and $118.4\,mg\,dm^{-3}\,h^{-1}$ based on the liquid volume) is around 2.5 times faster than the maximum value reported for a freely suspended cell system ($44.5\,mg\,dm^{-3}\,h^{-1}$ based on the liquid volume) and is at the same level as those reported for more complex systems.

CONCLUSIONS

The bead mill bioreactor developed during the course of this study is not only a simple and practical design but also demonstrates an excellent performance with respect to mass transfer and biodegradation of naphthalene and likely other hydrophobic hydrocarbons suspended in an aqueous phase. Mass transfer coefficients were found to be of similar magnitude to those reported for stirred

Table 1. Biodegradation rates of naphthalene in batch systems as reported in various studies (based on the volume of liquid in the reactor)

Reference	Microorganism	Bioreactor configuration	Physical state of the cells	Biodegradation rate ($mg\,dm^{-3}\,h^{-1}$)
20	*Pseudomonas* NGK1	Shake flask	Freely suspended cells	33.4–44.5
21	*Flavobacterium* sp	Slurry bioreactor	Freely suspended cells	0.7–2.5
22	*Sphingomonas aromaticivorans*	Two-phase partitioning bioreactor	Freely suspended cells	33[a]–85[b]
20	*Pseudomonas* NGK1	Shake flask	Immobilized in Ca alginate	21.4–38.1
20	*Pseudomonas* NGK1	Shake flask	Immobilized in agar	40–53.4
20	*Pseudomonas* NGK1	Shake flask	Immobilized in polyacrylamide	40–53.4
23	*Pseudomonas* NGK1	Shake flask	Immobilized in polyurethane foam	44.5–66.7
24	*Sphingomonas aromaticivorans*	Large-scale partitioning bioreactor	Freely suspended cells	119[c]
Present work	*Pseudomonas putida* ATCC 17 484	Bead mill bioreactor	Freely suspended cells	118.4

[a] Biodegradation rate of naphthalene in a mixture of naphthalene and phenanthrene (overall biodegradation rate of PAHs 40 $mg\,dm^{-3}\,h^{-1}$).
[b] Biodegradation rate of naphthalene in a mixture of naphthalene, phenanthrene, anthracene and acenaphthene (overall biodegradation rate of PAHs 95 $mg\,dm^{-3}\,h^{-1}$).
[c] Biodegradation rate of naphthalene in a mixture of naphthalene and phenanthrene (overall biodegradation rate of PAHs 238 $mg\,dm^{-3}\,h^{-1}$).

tank bioreactors, and were over 10-fold higher than the traditional roller bioreactor. Bioremediation rates for naphthalene particles were observed to reach $59.2\,mg\,dm^{-3}\,h^{-1}$ (on a total working volume basis) which is significantly superior to other bioreactors that utilize freely suspended cells and of similar magnitude to more complex bioreactor designs. To confirm the feasibility of the developed system for large-scale treatment of contaminated soils, mass transfer and biodegradation studies with soil-bound PAHs are currently underway.

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