Two-phase partitioning bioreactors: a new technology platform for destroying xenobiotics

Andrew J. Daugulis

Toxic organic compounds (xenobiotics) pose serious environmental and health risks worldwide. Biological treatment of these materials is severely constrained by their toxic and inhibitory nature and great care is required with respect to the rate at which they are provided to cells. The use of a second, distinct, organic phase in a bioreactor has been shown to provide a virtually foolproof means of feeding substrate to cells because this process concept relies only on thermodynamic equilibrium and the cells' own rate of metabolism. This technology can be applied to stockpiled xenobiotics as well as contamination of air, water and soil environments.

TECHNIQUES

The ecosphere continues to be challenged by the increasing amount and variability of toxic contaminants that are emitted by industrial activities. Such environmental contaminants are becoming more widespread as the pace of industrial activity accelerates, especially in developing countries, and also as a consequence of the difficulties in restricting the emissions of industrial activity from crossing national boundaries. The impact of these contaminants can be acute (e.g. arising from industrial mishaps, such as in Bhopal India in 1984, and the accidental discharge of toxins) or can be long-term through chronic exposure. Indeed, the link between immunological disorders (e.g. allergies and cancers) and environmental contamination is well known.

Among the most serious contaminants (both in terms of their impact and their resistance to treatment) are toxic organic compounds, particularly aromatic and halogenated compounds and the subset of these known as xenobiotics. Xenobiotic compounds are materials that are invariably man-made and are foreign to nature in the sense that they have been present in the ecosphere for relatively short periods of time and therefore efficient biodegradation pathways have not had adequate time to evolve. Consequently, the biological treatment of these materials is particularly challenging owing to the inhibition and/or toxicity of these compounds when they serve as microbial substrates.

In response to the inherent toxic nature of xenobiotic compounds, a process, rather than a microbial approach, has led to the development of an extremely promising technology for the treatment of toxic organic contaminants. This process is based on the use of two-phase partitioning bioreactors (TPPBs) and it will be argued that this approach can readily serve as a technology platform that, through simple modifications, can be used to treat contaminants of stored materials, as well as those that are present in air, water and soil environments.
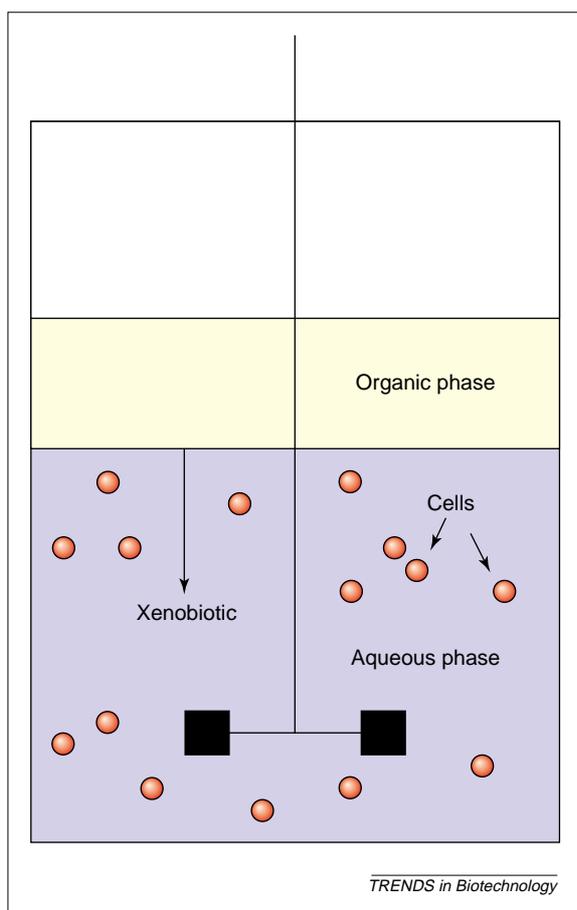
Two-phase partitioning bioreactors

In the biological treatment of xenobiotic compounds, the most significant challenge is substrate delivery. That is, addition of the substrate at too high a concentration will inhibit or even kill the organisms, whereas substrate addition at too low a rate will cause the cells to starve and result in a sub-optimal process performance. This situation is complicated by the fact that the substrate levels under consideration are extremely low – toxic levels of xenobiotic substrates can range from a few tens of milligrams per litre to a few hundred, so that precise and controlled delivery of these materials is exceedingly important. Conventional feedback control to achieve substrate delivery is not possible owing to the lack of probes (needed to measure substrate levels and compare them with the desired setpoint value) for the specific substrates in question and also because of the tendency of probes to drift – an unacceptable situation when miniscule variations in substrate concentrations can be lethal.

The TPPB concept[1] is based on the use of a water-immiscible and biocompatible organic solvent that is allowed to float on the surface of a cell-containing aqueous phase. The solvent is used to dissolve large concentrations of xenobiotic substrates (this is usually readily achievable because most organic contaminants are very hydrophobic), which then partition into the aqueous phase at low levels (determined by the partition coefficient of the compound in question). Thus, although very high amounts of toxic organic substrates can be added to a bioreactor, the cells experience only very low (sub-inhibitory) concentrations. Moreover, as the cells consume some of the substrate, disequilibrium is created, which causes more of the xenobiotic substrate to be partitioned into the aqueous phase as the system tries to maintain thermodynamic equilibrium. Thus, not only do appropriate amounts of xenobiotic substrates get delivered to the cells but

Andrew J. Daugulis
Dept of Chemical Engineering, Queen's University, Kingston, Ontario, Canada K7L 3N6.
e-mail: daugulis@ chee.queensu.ca

Organic phase

Cells

Xenobiotic

Aqueous phase

TRENDS in Biotechnology

also substrate delivery is ongoing (until the organic phase becomes completely depleted) and the rate is determined by the metabolic activity of the cells. As the total cell concentration increases, or as the cells become more adapted to the inhibitory substrate, the increasing demand for substrate is met by equilibrium partitioning. This represents cell-based process control in which the demand and supply of substrate are entirely driven by cellular processes. A schematic diagram of the TPPB process is shown in Fig. 1.

The TPPB concept has been demonstrated to be effective in degrading high levels of toxic organic compounds as seen by the examples summarized in Table 1. Most researchers have found that the xenobiotics themselves are sufficient to support microbial growth, with no need to provide additional substrates. In addition, most of these examples have been undertaken aerobically, although recent work in our laboratory on the degradation of explosives has been conducted under anaerobic conditions. Until now, bacteria have been the organisms of choice because they reproduce very quickly and can metabolize a wide spectrum of metabolites; we anticipate that higher organisms, such as fungi, will be used in the future. Although the citations in Table 1 are examples of demonstration of concept, only a few[1,6,9–13,16–18] have been arrived at after a systematic consideration of the interactions between

the xenobiotic substrate, the organic solvent and the cell. It is our belief that only through such a rigorous and systematic approach to process design will the TPPB concept be able to serve as a platform for widespread application in commercial situations. The ways in which actual remediation applications might present themselves and how the TPPB technology can be implemented to deal with them, will now be discussed.

Remediation of stored xenobiotics

Whether through the abandonment of containers of toxic organics as a result of industrial closures, the accumulation of banned chemicals or the stockpiling of materials that are past their best before date, there are large quantities of concentrated organics that need to be destroyed. These materials pose challenges to conventional biological treatment owing to their high concentrations and their highly toxic nature; however, this makes an ideal situation for the use of TPPBs.

Virtually all the examples listed in Table 1 describe the exogenous addition of toxic substrates to TPPBs, such as would be encountered in remediating stored xenobiotics. With a localized and relatively concentrated supply of xenobiotic materials, it has been shown to be relatively straightforward to add these substrates to the organic phase of a TPPB system, and have the cells consume the substrates, as described earlier. Additional complexities have also been handled by TPPBs, including the use of multiple xenobiotic substrates and repeated fed batch operation[9,10]. In the latter case, large amounts of xenobiotics can be destroyed on a semi-continuous basis at unprecedented rates. These studies have also shown the stability of the TPPB system with regard to microbial issues (no contamination or degeneration of the organisms) as well as solvent properties (no loss in biocompatibility, partition coefficient, etc.) over several weeks of operation. Optimization studies have also been undertaken to identify the most favourable feeding trajectory for consuming the maximum amount of xenobiotic in the shortest period of time by means of fed-batch operation[19,20]. Stored or stockpiled xenobiotics represent perhaps the most obvious example in which the TPPB concept can be used effectively, while also being one of the most challenging situations for conventional biological treatment methods. Current work in our laboratory is examining one such application, the destruction of energetic materials (explosives), which are in old munitions and have been stockpiled until destruction technologies become available.

Remediation of contaminated air

The waste gases produced during many industrial processes often contain low amounts of volatile organic compounds (VOCs), which are also xenobiotics (commonly compounds of the BTX group

Table 1. Recent examples of the use of TPPB systems for treating xenobiotics

Xenobiotic substrate	Organism	Solvent phase	Refs
Styrene	Mixed culture	Silicone oil	2
Phenanthrene (PAH)[a]	*Pseudomonas aeruginosa*	2,2,4,4,6,8,8-Heptamethylnonane	3
Naphthalene (PAH)	*Corynebacterium* sp.	Decane, dodecane, hexadecane	4
Various PAHs	Mixed culture	Silicone oil	5
2,4,6-Trichlorophenol	*Pseudomonas* sp.	Silicone oil	6
Dioxins	Mixed culture	Decane	7
Pentachlorophenol	*Arthrobacter* sp.	Diethyl sebacate	8
Phenol	*Pseudomonas putida*	2-Undecanone	9,10
Phenanthrene	*Pseudomonas* sp.	Silicone oil	11
Benzene, toluene	*Pseudomonas* sp.	Oleyl alcohol	12
Toluene, xylene	*Pseudomonas* sp.	Oleyl alcohol	13
BTX	*Pseudomonas* sp.	Oleyl alcohol	14
Various PAHs	Mixed culture	2,2,4,4,6,8,8-Heptamethylnonane	15
Benzene	*Alcaligenes xylosoxidans*	Hexadecane	16
Benzene	*Klebsiella* sp.	1-Octadecene	17
Benzene	*Alcaligenes xylosoxidans*	Hexadecane	18

[a]PAH, polyaromatic hydrocarbon.

– benzene, toluene and xylene). Although there are several physical and chemical means of removing these VOCs (e.g. carbon adsorption and incineration, respectively), VOC concentrations are often too low for these options to be economical, leaving biological treatment as the only viable option. The most common means of removing relatively low concentrations of VOCs from gas streams has been through the use of biofilters. Biofilters are usually cylindrical reactors that are packed with a substantially inert material (e.g. peat and wood chips), which provides an environment in which cells can grow. VOC-containing gas streams are forced up through these columns and the VOCs diffuse through a water layer (the beds are kept moist) to the adsorbed cells, where they are degraded. Although biofilters are effective in several situations, they have several key limitations[21,22]. First, the 'ripening' period of the biofilters during which cells proliferate to the point where the bed can be used is lengthy and is sometimes many days long. This prevents biofilters from coming on-line quickly and, in the event of a loss of cell activity, requires an additional start up period. Second, biofilters are usually restricted to the treatment of low concentrations of VOCs, typically <5 mg l^{-1}. This is partly owing to the fact that many VOCs are hydrophobic and are thus difficult to dissolve by the moist bed during the time that an element of gas spends traversing the bed, and also because the gas phase flows essentially in a plug flow manner through the bed, exposing the inlet sections to high (and potentially lethal) levels of VOCs. Should the first part of the bed lose its microbial population as a result of VOC lethality, no conversion of the VOCs will occur and the toxic slug of gas will continue up through the bed destroying the rest of the microbial population. Thus, biofilters are also susceptible to fluctuations (increases) in inlet VOC concentrations and, once the microbial population is lost, a new ripening period must be provided. Third, relatively speaking it is difficult to operate and control biofilters to achieve consistent performance. The bed must be kept moist and thus the ingoing air must be humidified. Moreover, because the continuous phase in this type of bioreactor is a solid, it is difficult to maintain uniform temperatures and pH. As the cells grow, the situation becomes even more problematic as void spaces become overgrown causing bed non-homogeneities to occur, which results in channelling and increased pressure drops across the beds.

As has been shown recently[16,17], the TPPB concept can be readily applied to the treatment of VOC-laden air streams with a very high degree of effectiveness. The key to using the TPPB system to treat gaseous organic contaminants is the presence, and use, of the organic solvent to trap the hydrophobic contaminant. In other words, the affinity for the VOC by the solvent is substantially higher (up to several orders of magnitude higher) than that exhibited by the modest amount of water present in biofilters. VOCs-trapping has been accomplished using an absorption column (with the solvent as the scrubbing liquid), which is connected in series to a TPPB. Once trapped by the solvent, the VOC substrate is sent to the TPPB system where it is transferred, on demand, from the solvent to the cells in the aqueous phase and the regenerated solvent is recirculated back to the absorber. Moreover, because the TPPB system is operated as a well-mixed system, the operational challenges experienced using biofilters (susceptibility to shock loads, maintaining uniformity of conditions, etc.) are

avoided. By controlling the type, amount and recirculation rate of solvent used for a particular VOC application it is possible to capture high concentrations of VOCs (to date, up to ten times higher than is possible with biofilters) and to deliver appropriate amounts to the cells for biodegradation. Thus, TPPBs can treat much higher levels of VOCs than can biofilters, as has been adequately shown, with equal or better removal efficiency, and maintain stable operation. The remediation of VOC-contaminated air by TPPBs has also been shown to possess significant process flexibility because the VOCs can be removed from the air stream by a solvent in a separate absorption column (well-mixed[16] or countercurrent[17]), or most recently in our laboratory by directly blowing the contaminated air into the TPPB itself, thus having a single unit for VOC capture and degradation.

Remediation of contaminated water

There are effective technologies for the treatment of wastewater effluents from industrial and municipal sources, which will not be addressed further here. However, in the event that spills of toxic organics (including xenobiotics) come into contact with water sources, new strategies must be developed. As a result of collisions and derailments involving the transportation of hazardous compounds, spills, and leakages from underground and aboveground storage tanks it is readily possible to imagine water sources, such as aquifers, becoming severely contaminated with organic materials. The level of contamination could include the aquifer becoming completely saturated with the organic chemicals (and therefore at a concentration many times higher than that compatible with conventional biodegradation), with possibly undissolved material also being associated with the aqueous plume. Given the hazardous nature of many xenobiotics, it is no longer permissible merely to let nature take its course and expect that the contamination will be either diluted to safe levels over time, or be eventually degraded by indigenous microorganisms. Rather, the contaminated water must be contained and treated.

Obviously, given the concentrations of toxic organics involved, this situation is incompatible with conventional biological treatment technologies. For example, if a water source were to become completely saturated with benzene, the benzene would be ~1700 mg l^{-1}, which is ~20–25 times the toxic limit of even the hardiest benzene-degrading organisms. Thus, unless a dilution of this magnitude (with a corresponding increase in volume of water to be treated) was used, the contaminated water would simply not be treatable using conventional means.

Recently, Yeom *et al.*[23] showed that a highly benzene-contaminated water source can be rapidly and effectively treated using TPPB technology. In this instance, 1 l of water at 1000 mg l^{-1} benzene was added to a bioreactor and a small volume (100 ml) of an appropriate, immiscible solvent (1-octadecene) was introduced to the bioreactor with mixing. The aqueous benzene concentration was thus reduced to 30–50 mg l^{-1}, at which time the system was inoculated with benzene-degrading organisms. The benzene was completely mineralized within 24 hours, at which time the aqueous phase was drained to 10% of its original volume (to act as an innoculum for a subsequent addition of highly contaminated water) and refilled with additional 1000 mg l^{-1} benzene-in-water solution. Because of its affinity for benzene, the solvent extracted the incoming benzene (preventing aqueous concentrations from reaching toxic levels) and subsequently released it to the cells on demand. Thus, in the present configuration, the solvent phase in the TPPB acted as a sponge, selectively extracting the toxic contaminant as it was introduced and releasing it in response to a declining aqueous-phase concentration caused by microbial benzene mineralization. This work also showed that, with the careful selection of a different solvent (limonene) with a higher partition coefficient for benzene, even smaller volumes of solvent (~68 ml, an amount that was not visible to the naked eye in the bioreactor) could be used to achieve similar performance.

In summary, the TPPB system readily treats highly contaminated water in a cyclic batch fashion, with repeated use of a small volume of solvent. No dilution of the highly toxic wastewater is required and it is anticipated that even the presence of undissolved xenobiotic along with the saturated aqueous phase could be readily handled by the use of a larger solvent volume.

Remediation of contaminated soil

Soil that has been contaminated with organic material usually requires treatment either because of the immediate effect that the contaminants have on the indigenous ecosphere or because of the possibility of migration to aquifers. Initially, two options are available for treating contaminated soil: *in situ* methods and *ex situ* methods in which the soil is removed and either treated and returned, or disposed of in a land-fill site. The *in situ* methods are biological and consist of biostimulation (in which non-carbon nutrients are provided to the contaminated soil in the hope that this will encourage autochthonous organisms to degrade the contaminant) and bioaugmentation (in which nutrients as well as targeted organisms are provided to the site). Although relatively inexpensive, these *in situ* methods have several limitations: (1) the rates of degradation are often relatively slow because environmental conditions (e.g. temperature, pH and moisture) are rarely optimal for mineralization to occur and are extremely difficult to control; (2) the slow rates create the concern that the contaminants could migrate away from the site before

Fig. 2. A 5 l TPPB system degrading polyaromatic hydrocarbons (PAHs), in which multi-gram quantities of PAHs are dissolved in the organic phase, delivering milligram quantities to the cells in the aqueous phase. The solvent phase is dodecane and the organism is *Sphingomonas* sp.

mineralization occurred; and (3) the processes are difficult to monitor, including the extent to which a target level of decontamination has actually been achieved.

There is a variety of *ex situ* methods of soil remediation including physical and chemical techniques (e.g. incineration). The most common *ex situ* biological method is soil washing, in which the contaminant is transferred to an aqueous phase, which is then sent to conventional biotreatment. A drawback of this approach is that many organic contaminants are often hydrophobic, requiring large volumes of water to be used (and subsequently treated). This difficulty is exacerbated by the fact that the organic content of the soil tends to bind the organic contaminants strongly, thus making efficient recovery of the contaminant by water washing difficult.

TPPB technology has been applied successfully to the treatment of soil that has been contaminated with xenobiotics. Two situations have been considered: direct content of the soil with an organic solvent that is subsequently used as the organic phase of a TPPB (Ref. 13) and an indirect method in which the organic contaminant is removed by volatilization and subsequently captured by the organic solvent in the TPPB and mineralized[25].

In the first scheme[13], a BTX spill was artificially carried out on sand and the sand was subsequently contacted with a solvent (oleyl alcohol), which also served as the second phase of a TPPB system. More

than 99% of the BTX was removed through one contact with the solvent and the residual solvent was removed from the sand by a water wash (oleyl alcohol and water are mutually insoluble), which was used as the aqueous phase of the TPPB. The BTX was rapidly mineralized in the TPPB and the solvent was used successfully for a second time to treat another artificial spill. Work is under way in our laboratory aimed at removing polyaromatic hydrocarbons (PAHs) from contaminated soil (including clay and soil that contains organics) by means of solvent extraction and then using the solvent as the second phase in a TPPB. Given that PAHs are sparingly soluble in water but are easily removed by contact with solvents, we have been able to readily decontaminate soil containing high levels of PAHs using small solvent volumes (A. Daugulis, unpublished observations). In this application, careful solvent selection is particularly crucial because the solvent must have appropriate physical and chemical properties related to PAH extraction and also meet the biological constraints posed by the TPPB. A photograph of a 5 l TPPB in which PAHs are being mineralized is shown in Fig. 2.

TPPBs are also particularly effective in treating contaminated soil when the contaminants are relatively volatile[24]. In this instance, a well-known means of volatilizing contaminants from soil, soil vapour extraction (SVE) (Ref. 25) was used. SVE involves blowing air through contaminated soil (either *ex situ* in a containment device or *in situ* by sinking bore holes and gas collection pipes) to desorb the volatile contaminant, followed by a step (such as activated carbon adsorption) to trap the material. SVE has been used extensively, particularly for reclaiming soil beneath defunct gasoline stations. In a recent study[25], soil with high organic content was amended with a hazardous gasoline component (benzene) and placed in a flask. Air was then blown through the flask (mimicking SVE), volatilizing the benzene and was then sparged directly into a TPPB system. Because the benzene was now in the gas phase, the TPPB operated in a manner similar to that described above. A highly contaminated sample of soil (equivalent to a contamination level of 11 kg benzene m^{-3} soil) was rapidly treated in this fashion, with 93% of the benzene being stripped from the soil, of which 91% was degraded, in 2 hours. The high concentrations and masses of benzene (especially initially) in the gas stream leaving the soil sample were found to be far too high to be handled by either a biofilter or a conventional wastewater treatment system. As has been seen before for other applications, the solvent in the TPPB acted as a sponge with high affinity for the organic contaminant, capturing it and then releasing it, at sub-inhibitory levels, on demand to the cells in the TPPB. Treatment of volatile contaminants in soil in this fashion has several advantages, including the fact that the soil never comes into direct contact with

the contents of the TPPB, thereby ensuring that neither the soil nor the TPPB are compromised.

Conclusion

As was seen in Table 1, the TPPB concept has been demonstrated to be effective at degrading a variety of toxic substrates. The process is based on the fundamental principle of thermodynamic equilibrium, in which a molecule will spontaneously partition from one phase to another based on its partition coefficient in the ternary system. The TPPB technology is also self-regulating in the sense that the metabolic rate of the microorganisms determines the feeding rate of substrate. As has been seen, the TPPB concept can be applied to the treatment of high concentrations of stored xenobiotics, as well as xenobiotic contaminants in gas streams, water and soil. There are three key issues that make the TPPB concept effective and that will determine its commercial use. First, as long as an organism can be found that degrades a particular contaminant, the TPPB system can be used. Such organisms can be isolated, obtained from culture collections, and/or generated using genetic manipulation. Second, the TPPB system consists of a conventional stirred tank bioreactor. This not only makes the system attractive from the standpoint of

being able to easily control reactor conditions at desired levels, but also provides for simple procurement and scale up. Third, most xenobiotics are highly soluble in organic solvents and substantially less so in water, which makes the TPPB system extremely effective at trapping or recovering xenobiotics from various sources.

Although there are many xenobiotic compounds that require effective means of destruction, there are also many organic solvents that can be used in TPPB systems. Commercial applications will require tailoring a solvent to meet the needs of each particular situation. This will involve a rational and systematic approach to solvent selection, taking into account the physical, chemical and biological criteria imposed by the xenobiotic and organism under investigation, as well as practical issues such as cost, safety, and so on. Certainly not all of the examples of the use of TPPBs listed in Table 1 adhered to this principle, seemingly having used a solvent that happened to work; thus some of the references are more of scientific rather than of practical interest. Systematic solvent selection methods have been well defined[26] and were used in several of the key references cited and their widespread adoption will be important to the commercial success of TPPB technology.

References

1 Collins, L.D. and Daugulis, A.J. (1996) Use of a two phase partitioning bioreactor for the biodegradation of phenol. *Biotechnol. Tech.* 10, 643–648

2 Al Aalam, S. *et al.* (1993) High efficiency styrene biodegradation in a biphasic organic/water continuous reactor. *Appl. Microbiol. Biotechnol.* 39, 696–699

3 Kohler, A. *et al.* (1994) Enhanced biodegradation of phenanthrene in a biphasic culture system. *Biodegradation* 5, 93–103

4 Gamerdinger, A.P. *et al.* (1995) Effect of aliphatic nonaqueous phase liquids on naphthalene biodegradation in multiphase systems. *J. Environ. Qual.* 24, 1150–1156

5 Vannek, P. *et al.* (1995) Biodegradation of polycyclic aromatic hydrocarbons in a two-liquid-phase system. In *Bioremediation of Recalcitrant Organics* (Vol. 7) (Hinchee, R.R. *et al.*, eds) pp. 55–62, Battelle Press

6 Ascon-Cabrera, M. and Lebeault, J-M. (1995) Cell hydrophobicity influencing the activity/stability of xenobiotic degrading microorganisms in a continuous biphasic aqueous-organic system. *J. Ferment. Technol.* 80, 270–275

7 Barkovskii, A. and Adriaens, P. (1995) Biological and abiotic dechlorination of highly chlorinated dioxins in biphasic microcosms. In *Bioremediation of Recalcitrant Organics* (Vol. 7) (Hinchee, R.R. *et al.*, eds), pp. 73–82, Battelle Press

8 Munro, D.R. and Daugulis, A.J. (1997) The use of an organic solvent and integrated fermentation for improved xenobiotic degradation. *Resource Environ. Biotechnol.* 1, 207–225

9 Collins, L.D. and Daugulis, A.J. (1997) Biodegradation of phenol at high initial concentrations in two phase partitioning batch and fed batch bioreactors. *Biotechnol. Bioeng.* 55, 155–162

10 Collins, L.D. and Daugulis, A.J. (1997) Characterization and optimization of a two-phase partitioning bioreactor for the biodegradation of phenol. *Appl. Microbiol. Biotechnol.* 48, 18–22

11 Bouchez, M. (1995) Substrate availability in phenanthrene biodegradation: transfer mechanism and influence on metabolism. *Appl. Microbiol. Biotechnol.* 43, 952–960

12 Collins, L.D. and Daugulis, A.J. (1999) BTX degradation part I: solvent selection and toluene degradation in a two-phase partitioning bioreactor. *Appl. Microbiol. Biotechnol.* 52, 354–359

13 Collins, L.D. and Daugulis, A.J. (1999) BTX degradation part II: effect of substrate interactions and feeding strategies in toluene-benzene and toluene-p-xylene fermentations in a partitioning bioreactor. *Appl. Microbiol. Biotechnol.* 52, 360–365

14 Collins, L.D. and Daugulis, A.J. (1999) Simultaneous biodegradation of benzene, toluene and p-xylene in a two phase partitioning bioreactor: concept demonstration and practical application. *Biotechnol. Prog.* 15, 74–80

15 Marcoux, J. *et al.* (2000) Optimization of high-molecular-weight polycyclic aromatic hydrocarbons' degradation in a two-liquid-phase bioreactor. *J. Appl. Microbiol.* 88, 655–662

16 Yeom, S.H. and Daugulis, A.J. (2001) Development of a novel bioreactor system for the treatment of gaseous benzene. *Biotechnol. Bioeng,* 72, 156–165

17 Yeom, S.H. *et al.* (2000) Treatment of high-concentration gaseous benzene streams using a novel bioreactor system. *Biotechnol. Lett.* 22, 1747–1751

18 Yeom, S.H. and Daugulis, A.J. (2001) Benzene degradation in a two-phase partitioning bioreactor by *Alcaligenes xylosoxidans* Y234. *Proc. Biochem.* 36, 765–772

19 Cruickshank, S.M. *et al.* (2000) Dynamic modelling and optimal fed-batch strategies for a two-phase partitioning bioreactor. *Biotechnol. Bioeng.* 67, 224–233

20 Cruickshank, S.M. *et al.* (2000) Modeling of a continuous two-phase partitioning bioreactor for the degradation of phenol. *Proc. Biochem.* 35, 1027–1035

21 Hodge, D.S. and Devinny, J.S. (1995) Modelling removal of air contaminants by biofiltration. *J. Environ. Eng.* 121, 21–32

22 Shareefden, Z. and Baltzis, B.C. (1994) Biodegradation of toluene vapor under steady-state and transient conditions: theory and experimental results. *Chem. Eng. Sci.* 49, 4347–4360

23 Yeom, S.H. *et al.* The use of partitioning bioreactors for the treatment of high-concentration benzene solutions. *Can. J. Chem. Eng.* (in press)

24 Yeom, S-H. and Daugulis, A.J. (2001) A two-phase partitioning bioreactor system for treating benzene-contaminated soil. *Biotechnol. Lett.* 23, 467–473

25 US Environmental Protection Agency (US EPA) (1991) *Soil Vapor Extraction Technology-Reference Handbook*, EPA-540/2–91/003, Washington, DC

26 Bruce, L.J. and Daugulis, A.J. (1991) Solvent selection strategies for extractive biocatalysis. *Biotechnol. Progr.* 7, 116–124