Bioprocess Biosyst Eng (2006) 29:229–240
DOI 10.1007/s00449-006-0071-2

ORIGINAL PAPERS

Benzene vapor treatment using a two-phase partitioning bioscrubber: an improved steady-state protocol to enhance long-term operation

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Received: 24 May 2006 / Accepted: 29 May 2006 / Published online: 29 August 2006

Abstract The performance and stability of a two-phase partitioning bioscrubber (TPPB) containing 33% (vol.) *n*-hexadecane as an immiscible phase was investigated during 30 days of continuous gaseous benzene treatment. Elimination capacities of 141 ± 12 g/m^3 h were achieved by *Achromobacter xylosoxidans* Y234 while maintaining >99% removal throughout. A new steady-state operating strategy that limits excessive biomass production by directing substrate consumption to maintenance energy has eliminated the requirement for frequent exchange of liquid contents. Simplifying the operating protocols in this manner has dramatically reduced material costs and rendered the TPPB operational requirements as more comparable (in terms of frequency of required operator inputs) with other vapor-phase bioreactors. The practicality of the proposed simplification to the operating protocol was confirmed by demonstrating that intermediate metabolites were not accumulating in the TPPB, inorganic nutrient requirements were readily predictable, and that high culture viability could be sustained for prolonged cell retention times (30 days).

Keywords Bioscrubber · Biofiltration · Partitioning bioreactor · Benzene

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Introduction

Vapor-phase bioreactors represent an increasingly attractive alternative to physicochemical processes for the treatment of waste gases contaminated with volatile organic compounds (VOCs) due to their generally lower capital and operating costs [1]. However, the efficacy of more traditional bioreactor configurations, such as biofilters and trickling biofilters, is often hindered by inherent design limitations which in turn confine their application to the treatment of only very dilute waste gases. Common problems encountered in these designs include airflow channeling, drying of the filter bed, low rates of substrate and oxygen mass transfer, and inhibition by toxic substrates [1]. Two-phase partitioning bioscrubber (TPPB) technology was originally adapted from its bioreactor predecessors [2–4] to address these limitations, allowing it to pursue alternative opportunities treating more highly concentrated waste gases which can typically only be performed using physicochemical methods. A TPPB is comprised of an aqueous phase, containing cells and inorganic nutrients, as well as an immiscible (typically organic) phase which sequesters high concentrations of toxic substrates away from the cells through equilibrium partitioning. Careful selection of a suitable organic phase ensures it to be biocompatible, non-bioavailable, immiscible with the aqueous phase, and non-volatile [5], while also enhancing VOC absorption rates for compounds with high Henry's Law coefficients [6]. The rate of partitioning transfer between the two liquid phases has been shown to be rapid enough that it is controlled by the metabolic activity of the cells [7], allowing high levels of bioactivity to be sustained when treating elevated loads of toxic substrates.

The original TPPB design of Yeom and Daugulis [7] coupled a benzene absorption column with a separate, continuous two-phase partitioning bioreactor. The *n*-hexadecane organic phase circulated between the column, where benzene was absorbed from the waste gas, and the partitioning bioreactor where it was ultimately consumed. Low agitation rates were employed to limit phase dispersion, resulting in slow rates of oxygen transfer and benzene partitioning which perhaps controlled the overall biodegradation rate. Davidson and Daugulis [8] improved upon this design through process compression using a single vessel, thus amalgamating the substrate capture and biodegradation steps. The bioscrubber could then be aggressively mixed, promoting higher rates of absorption and partitioning of both oxygen and benzene. The liquid contents of their TPPB were no longer continuously replenished; instead medium exchanges were employed, in which 50% of the bioscrubber liquid contents were replaced with fresh sterile aqueous medium and organic phase, were performed approximately every 20 h to restrict biomass levels between 2 and 4 g-CDW/L. Though not demonstrated beyond 6 days, it was concluded that operation in this manner could likely be fostered perpetually however, the high frequency of medium exchanges would prove quite labor intensive and generate significant waste (biomass, and effluent aqueous and organic phases) requiring disposal or regeneration.

Alternatively, Andrews and Noah [9] hypothesize that in a continuous bioreactor with a limited supply of carbon and energy source, as the mean cell residence time becomes infinite, the culture will eventually approach a condition under which all of the contaminant must be used to satisfy the culture's total maintenance energy requirements. The proposed concept of a 'maintenance state' has previously been demonstrated in chemostat cultures that used microfiltration to remove cells from the effluent liquid stream, retaining them to eventually achieve a stable steady-state biomass concentration [10, 11]. It has also been demonstrated that a steady-state biomass concentration can be attained in the TPPB by forgoing medium exchanges and retaining its liquid contents [12]. However, it is still unclear as to whether this steady state could be sustained over long periods without inducing process instabilities which could arise due to accumulation of toxic metabolites or depletion of an essential nutrient. If a steady state culture operating in maintenance mode can in fact be sustained in a TPPB, the requirement for medium exchanges to control the biomass concentration could be eliminated translating into reduced waste and material requirements and greatly simplifying the routine operator requirements. As Diks et al. [13] note, the stability and reliability of emerging vapor-phase bioreactor technologies should be guaranteed during long-term applications in order to compete with conventional technologies. With this mind, the underlying objective of this study was to demonstrate that high benzene elimination capacities could be both achieved and maintained in a TPPB over a 30 day (720 h) period without the need for arduous medium exchanges, by promoting steady-state operation with a culture in maintenance mode. In order to rationally eliminate medium exchanges from the operating protocol, several preliminary experiments were performed to investigate other pertinent phenomena which could potentially contribute to the overall demise in performance in the absence of medium exchanges so as to ensure that process stability would not be sacrificed. These include: (1) the possible accumulation of intermediate metabolites and overall benzene biodegradation potential of *A. xylosoxidans* Y234, (2) prediction of inorganic nutrient requirements, and (3) assessment of cellular viability during the various anticipated growth stages (i.e., actively growing versus steady-state maintenance cultures) and as a function of the cell residence time. By direct comparison with the medium exchange protocols followed by Davidson and Daugulis [8], we aim to conclusively demonstrate that the proposed steady-state operation will result in greatly reduced material and operating requirements relative to previously followed protocols.

Materials and methods

Microorganism and media

Achromobacter xylosoxidans Y234, a known degrader of benzene, was cultivated from cryogenically preserved stock in four 125 mL Erlenmeyer shake flasks, each containing 50 mL of enrichment medium (A) (Table 1), incubated at 30°C, and agitated at 150 rpm for 24 h prior to their inoculation in the bioscrubber.

Detection of intermediates

Identification of metabolic intermediates was undertaken using a Biochrom Ultrospec 3000 UV/Visible Spectrophotometer to identify characteristic absorbance peaks of potential metabolite compounds between wavelengths of 200–600 nm. Experiments were performed in three quartz cuvettes, each containing 2 mL of the original medium (B) supplemented with

Table 1 Media formulations used in this study

Nutrient	Enrichment medium (A)	Original medium (B)	Modified medium (C)
$(NH_4)_2SO_4$ (g/L)	7	14	28
$MgSO_4 \cdot 7H_2O$ (g/L)	0.75	1.5	1.5
K_2HPO_4 (g/L)	6.6	13.2	6.6
KH_2PO_4 (g/L)	8.42	16.84	8.42
Trace elements (mL/L)	1	2	2
Sodium benzoate (g/L)	2	–	–

Trace element solution (each in g/L): $FeCl_3 \cdot 6H_2O$, 16.2; $CaHPO_4$, 9.44; $CuSO_4 \cdot 5H_2O$, 0.15; citric acid, 40

100 mg/L catechol as the sole carbon and energy source. Catechol (P_{vap} = 0.03 mmHg at 20°C), the common first intermediate product in the aerobic degradation of benzene [14], was selected as the initial substrate to limit the volatile losses that would have occurred had benzene (P_{vap} = 75 mmHg at 20°C) been used instead. The first cuvette served as an abiotic control, while the second was inoculated with 0.1 mL of a washed cell suspension with a cell density of approximately 12 g-CDW/L. The cell suspension was generated from a culture that was pre-adapted for benzene degradation in an aqueous bioscrubber to which was fed a dilute benzene feed for 3 days. Samples were then drawn and centrifuged to remove the aqueous supernatant before washing of the biomass pellet with sterile, carbon-free medium. The final cuvette was inoculated with 0.1 mL of the cell-free aqueous supernatant solution obtained during the preparation of the pre-adapted cell suspension. The spectra of each cuvette were scanned periodically between wavelengths of 200–600 nm to monitor for absorbance peaks. Samples from subsequent batch experiments, as well as from the TPPB during continuous treatment were regularly analyzed for absorbance peaks within the same spectral region to detect for the possible accumulation of these intermediate compounds.

In a subsequent experiment intended to further search for benzene degradation intermediates and by-products, a 6 L New Brunswick BioFlo® III bioreactor containing 2.7 L of original medium (B) and 0.3 L of *n*-hexadecane (10% vol.) was operated as a closed batch partitioning bioreactor by sealing all of the orifices (with Teflon) to eliminate volatile losses. Since this sealed bioreactor could not be aerated, the aqueous phase was first saturated with 1 atm dissolved oxygen. Liquid benzene was then added to the bioreactor, which rapidly partitioned between the gas and liquid phases, together with inoculation using pre-adapted cells just prior to sealing the bioreactor and initiation of sampling. As benzene degradation occurred, liquid samples were periodically drawn from bioreactor to detect for the presence of possible intermediate metabolites as indicated by characteristic absorbance peaks. The benzene concentration in the bioreactor headspace was also periodically measured by gas chromatography and used together with previously validated phase equilibrium assumptions (data not shown) to determine the benzene content in the aqueous and organic phases. After benzene was no longer detectable in the headspace, organic phase samples were directly analyzed to confirm that benzene was no longer present in the bioreactor.

Aqueous inorganic nutrient measurement

During continuous operation of the TPPB (as described below) inorganic nutrient levels were measured in the aqueous fraction of liquid samples after centrifugation. Ammonium-N levels were determined using an ammonia selective electrode (Orion 95–12) attached to an Orion research electrode meter (Expandable ionAnalyzer EA 920). After shifting the sample to at least pH 13 via addition of a small volume of 6 M KOH (9:1 sample:base), the mixture was allowed 1 min to equilibrate prior to measurement and comparison to an external calibration. Analysis of other inorganic elements of interest (Mg, K, P, S) was performed using a Varian Vista AX simultaneous inductively coupled plasma atomic emission spectrometer (ICP-AES), following digestion of aqueous samples with 2% (vol.) nitric acid. Nitrate and nitrite ions were also analyzed using ion chromatography. Analysis was performed using a Dionex DX-300 gradient chromatographic system equipped with an IonPac AS11 (4 × 250 mm) analytical column and an ASRS Ultra II 4 mm ion suppressor column. Sample output was calibrated using stock solutions of potassium nitrate and sodium nitrite.

Benzene measurement

Benzene concentrations in the organic and gas phases were measured using a Perkin Elmer AutoSystem gas chromatograph fitted with a flame ionizing detector and a fused silica capillary column (DB-5, 0.53 mm I.D., 30 m length, 1 μm film thickness, Model 125–503J, J&W Scientific, Inc., USA) and helium carrier gas flowing at 30 mL/min. The injector, column, and detector temperatures were set at 180, 150, and 280°C, respectively for gaseous analysis, while the column temperature was programmed to increase from 60 to 280°C at 45°C/min when sampling the organic phase.

The output was converted to concentrations using an external calibration and aqueous-phase benzene concentrations were estimated using a previously determined experimental partitioning coefficient, assuming an equilibrium relationship with the organic phase.

Biomass measurement

Biomass concentrations were determined through optical density measurements at 650 nm using a Biochrom Ultrospec 3000 UV/Visible Spectrophotometer (Biochrom Ltd., UK) and compared to a cell dry weight (CDW) calibration. Liquid supernatant was removed after centrifugation and the biomass was resuspended in deionized water to its original concentration and serially diluted as required. Cell enumeration was also performed throughout the experiment by a plate count technique. After serial dilution of the bioscrubbers liquid contents in sterile, carbon-free medium, samples were cultured on solid agar plates, composed of enrichment medium (A) supplemented with 15 g/L Bacto agar and substituting 2 g/L glucose for sodium benzoate, then incubated at 30°C for 24 h in order to enumerate viable cell counts.

Bioscrubber operation and sampling procedures

A 6 L New Brunswick BioFlo® III bioreactor served as the bioscrubber, containing 2 L of aqueous medium and 1 L *n*-hexadecane, constituting an organic volume fraction of 0.33. The two compositions of aqueous nutrient medium used in bioscrubber in this study are presented in Table 1. The bioscrubber was agitated with two Rushton turbines at 800 rpm and aerated at a total rate of 60 or 75 L/h (benzene feed plus make-up aeration), corresponding to 0.33 or 0.42 vvm, respectively, based on the total volume of 3 L. Conditions were automatically maintained at 30°C, and at pH 6.6 by adding 6 M KOH. Dissolved oxygen levels were measured with a polarographic-membrane probe (Broadley and James Corp., USA) and automatically recorded with a computer interface. A benzene-rich gas stream was produced by bubbling sterile air through a 2 L flask containing 1.5 L of liquid benzene maintained at 30°C in a water bath. This air stream was then mixed with a second sterile make-up air stream (used to supply additional oxygen) prior to entering the bioscrubber through a sparger mounted at the bottom of the vessel. Flow rates of the two streams were individually controlled via separate rotameters whose relative actuation allowed for fine-tuning of the desired influent benzene concentration after mixing of these streams. Liquid samples of the completely-mixed reactor contents were drawn periodically into 15 mL centrifuge tubes prior to centrifugation for 15 min at 4°C and 3,400 rpm to separate the solid, aqueous, and organic phases for individual analyses.

Results

Formation of intermediate metabolites

The benzene degradation potential of *A. xylosoxidans* Y234 and the formation of the intermediate products associated with its metabolic pathway were investigated using absorption spectra. Abiotic analysis of the control cuvette indicated that catechol has a characteristic peak at approximately 275 nm which did not diminish over the course of the 19 h experiment. In the cuvette containing acclimated *A. xylosoxidans* Y234 cells, however, the formation of a distinct peak between 376–379 nm occurred almost instantly, reaching a maximum value of 2.3 AUs in just 10 min. The amplitude of this peak was sustained through 45 min, thereafter slowly declining and reaching 2.1 AUs by 105 min. Concurrently, the solution took on a yellow coloration which rapidly increased in intensity before stabilizing. The samples were subsequently left overnight and after 19 h no peak was observed between 376–379 nm, the yellow coloration had disappeared, and the peak at 275 nm (catechol) had greatly diminished. No other peaks were observed in the spectra at any other time throughout this experiment. The appearance of an intermediate peak between 376–379 nm is typically attributed to the formation of 2-hydroxymuconate semialdehyde (2-HMS), the *meta*-cleavage product of catechol [15]. This result is also supported by the findings of Gibson et al. [16] who attributed yellow coloration during benzene degradation to the formation of 2-HMS. On the other hand, the *ortho*-cleavage product, *cis,cis*-muconate, has a characteristic absorbance at 255 nm [17], and no intermediate peaks were formed near this wavelength. The apparently slow rate of catechol degradation was likely a result of the limitation of oxygen in the cuvette, and is not representative of the conditions in a bioreactor or bioscrubber. However, since the peak corresponding to what is speculated to be 2-HMS did eventually disappear, the evidence suggests that this is not a terminal end-product, but simply an intermediate metabolite which accumulated because its subsequent degradation step was rate limiting in the pathway. Finally, addition of cell-free aqueous supernatant to the solution resulted in no loss of absorbance at 275 nm, nor formation of any other peaks within the spectrum, indicating

that catechol 2,3-dioxygenase, which catalyzes the suspected *meta*-cleavage pathway of catechol, was not present in the aqueous solution.

A subsequent sealed batch partitioning bioreactor experiment confirmed the appearance of a peak at about 375 nm associated with the degradation of benzene, as shown in Fig. 1, which was also coupled with the appearance of a yellow coloration in the medium. Both were observed to disappear within 2 h, shortly after benzene could no longer be detected in the system. These results indicate that although intermediate metabolites will be present, accumulation of terminal end-products in the bioscrubber should not be anticipated during continuous benzene degradation, making their would be removal by medium exchange unnecessary.

Inorganic nutrient requirements and cellular viability

To better understand the nutritional requirements of the culture, a TPPB was operated until its performance was ultimately disrupted as the result of the depletion of an essential inorganic nutrient, all the while monitoring aqueous nutrient levels. This experiment was also used as an opportunity to assess the ability to sustain a viable culture as the cell retention time is increased. Original medium (B) comprised the aqueous phase of the bioscrubber which was provided with 60 L/h of gaseous benzene feed at a concentration

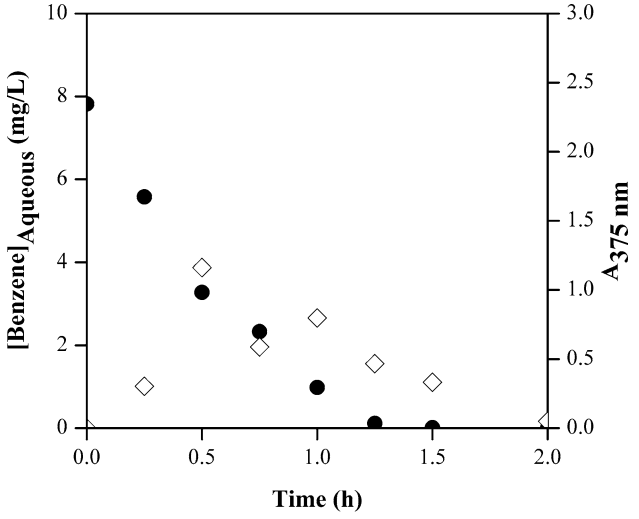

Fig. 1 Degradation of benzene is measured by the aqueous phase concentration (*solid circles*) in a sealed two-phase partitioning bioreactor containing 10% (vol.) *n*-hexadecane and associated production and gradual consumption of an unidentified intermediate metabolite, as measured by absorbance at 375 nm (*open diamonds*)

averaging 3.9 ± 0.5 mg/L (average loading rate of 232 ± 30 mg/h). The results are depicted in Fig. 2a–c. After a brief lag phase (not shown) the benzene removal approached 99% efficiency, constituting an elimination capacity of 77 ± 10 g/m^3 h which was sustained for a period of 500 h apart from a 24 h period beginning at 271.5 h when the influent benzene concentration was roughly doubled to 7.6 ± 0.4 mg/L to observe the dynamic response of the culture and its associated nutrient requirements. During this transient period, removal efficiencies also remained above 99% and the elimination capacity achieved 151 ± 7 g/m^3 h. Although the bioscrubber developed a yellow color, spectrophotometric measurements throughout the experiment did not reveal any peaks other than that corresponding to benzene between 200 and 600 nm, indicating that intermediates were not accumulating above the detection limit of the instrument.

Throughout the first 70 h of operation, biomass levels accumulated in the bioscrubber in a linear manner which is indicative of the mass transfer limitation experienced by the process. The average rate of biomass production measured during this period was 107.3 ± 0.8 mg/h and the average rates of N and P assimilation were 21.4 ± 3.7 and 4.2 ± 1.4 mg/h, respectively, while rates of Mg, K, and S uptake were observed to be negligible. Biomass accumulation rates slowly declined and, after approximately 170 h, the biomass approached a steady-state concentration of 6.8 ± 0.3 g/L. However, even though no additional biomass was accumulating during the steady-state portion of operation, N was still being depleted at a rate of 6.8 ± 1.0 mg/h. By this point, discernable trends regarding the uptake of P, K, S, and Mg could no longer be deduced with any degree of confidence. However, it was clear from the data that ample amounts of these inorganic nutrients still remained in the bioscrubber throughout the duration of the experiment. Measurement of the number of viable cells by plate count began after approximately 100 h as the biomass began to approach its steady-state concentration and, as seen in Fig. 2b, indicated that the number of viable cells in the bioscrubber remained relatively constant throughout the remainder of the experiment, demonstrating no apparent loss in viability. During the transitory period in which the influent concentration was spiked for 24 h, excess substrate became available and biomass again accumulated from its previous steady-state value at an average rate of 191 ± 19 mg/h while N consumption increased to 33.6 ± 6.4 mg/h. After this brief transient, biomass levels declined slightly as a result of expected endogenous activity before again achieving a constant concentration of

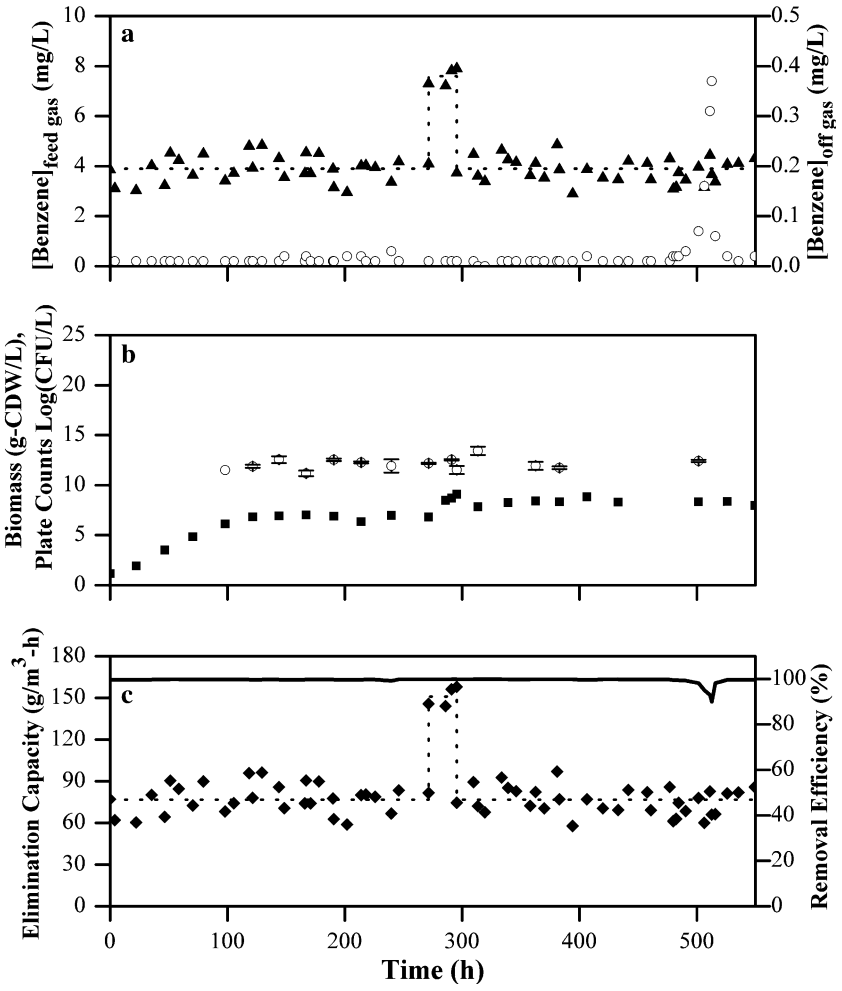

Fig. 2 Bioscrubber performance during the preliminary experiment, including response to a 24 h, twofold step increase in benzene feed concentration: **a** influent (*solid triangles*) and effluent (*open circles*) gas benzene concentrations. The average influent benzene concentration of 3.9 ± 0.5 mg/L (*dashed line*) is also shown. **b** Biomass concentration (*solid squares*) and plate counts (*open circles*) with error bars at one standard deviation from triplicate measurement. **c** Removal efficiency (*solid line*) and elimination capacity (*solid diamonds*). The average elimination capacities obtained under nominal and transient operation of 77 ± 10 g/m^3 h and 151 ± 7 g/m^3 h (*dashed line*), respectively, are also shown

8.4 ± 0.2 mg/L. N consumption again continued at a slow rate, averaging 3.7 ± 0.5 mg/h without any apparent biomass accumulation. While this value is significantly different than that originally measured during steady-state operation, it is much lower than that which was measured to be associated with periods of significant accumulation of biomass.

The performance of the bioscrubber declined sharply after approximately 490 h, at which point benzene rapidly accumulated in both liquid phases as well as the effluent gas stream (Fig. 3). Analysis of the aqueous nutrient levels indicated that this precipitous decline in removal efficiency was associated with the complete depletion of ammonium-N from the medium. To replenish levels of this essential nutrient and attempt to revive performance, 5 g $(NH_4)_2SO_4$ in 50 mL of sterile water was added to the bioscrubber at 513 h. As can be seen, removal rates were quickly restored and pre-limitation performance was attained in about 12 h. Dissolved nitrate and nitrite levels measured at the time of depletion were found to be no different than those measured at the start of the experiment, indicating that nitrification did not contribute to ammonium depletion. By the time of N limitation, 115 ± 15 g of benzene had been removed by the bioscrubber, translating into a C/N mass ratio of approximately 20. The possibility of volatile nutrient losses (particularly N-ammonia) via air stripping was later investigated using an identical, cell-free bioscrubber arrangement which was aerated for 200 h while monitoring dissolved inorganic nutrient levels and losses were found to be negligible. To verify the predicted N requirement, a second bioscrubber experiment was performed using the same initial inorganic nutrient composition, however, at a higher benzene loading rate. It was anticipated that the associated rates of inorganic nutrient uptake would proportionally increase, leading to nitrogen limitation much earlier. A constant benzene loading rate averaging 451 ± 40 mg/h was applied to the bioscrubber and an average elimination capacity of 150 ± 13 g/m^3 h was achieved, maintaining over 99% removal efficiency. After approximately 240 h,

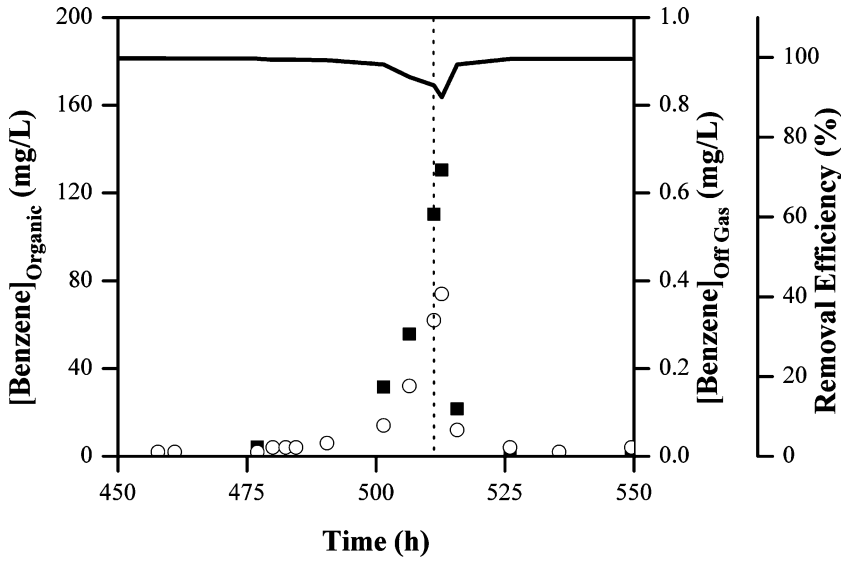

Fig. 3 Response of the nitrogen-limited TPPB to the addition of 5 g $(NH_4)_2SO_4$ as indicated by the benzene concentration in the organic phase (*solid squares*) and off-gas (*open circles*), as well as instantaneous removal efficiency. *Dashed line* at 513 h indicates point of addition

the performance of the bioscrubber began to decline, as evidenced by decreasing benzene elimination capacities and removal efficiencies. Aqueous measurements confirmed that ammonium-N that had once again been depleted, by which time approximately 108 ± 9 g of benzene (100 ± 9 g of benzene-C) had been removed by the bioscrubber for growth and energy purposes. This amount is very close to the total quantity of benzene-C removed in the preliminary experiment, even though it occurred over 260 h sooner, and again corresponds to a C/N ratio of about 19. These results indicate that the previously observed deactivation due to ammonium-N depletion was independent of both the culture age and the particular operating conditions selected.

Long-term operation

Since the preliminary experiments indicated that there would be no expected accumulation of inhibitory intermediates nor significant loss in cellular viability, and that nutrient requirements were now more easily predicted, an attempt at stable operation for 30 days without medium exchange was made. Analogous conditions to those used by Davidson and Daugulis [8] were selected so that the proposed steady-state approach could be directly compared to their medium exchange protocol. Davidson and Daugulis [8] operated a 1 L bioscrubber to treat benzene waste gases with *A. xylosoxidans* Y234 using 33% (vol.) *n*-hexadecane at 30°C, pH 6.6, 800 rpm, 0.42 vvm, and an aqueous phase containing half the nutrient concentration of the original medium (B) used here (Table 1). A lower initial nutrient concentration could be used in their study because fresh nutrients were re-supplied every 20 h throughout the course of the experiment by medium exchange. In order to maintain the same gas residence time and benzene loading capacity between the two studies (0.42 vvm and 140 g/m^3 h, respectively), the total gas flow rate and the target benzene mass loading rate in the present study were scaled to 75 L/h and 420 mg/h, respectively, to account for the larger liquid volume now employed. In both studies, these conditions correspond to an average benzene feed concentration of 5.6 mg/L after mixing with make-up air.

Since the nutritional analysis identified N as the single limiting component while all other nutrients remained in excess, the aqueous medium was accordingly reformulated as the modified medium (C), listed in Table 1. K_2HPO_4 and KH_2PO_4 concentrations were decreased while the initial concentration of $(NH_4)_2SO_4$ was doubled relative to that used in the original medium (B). According to the predicted C/N ratio of 20, this initial ammonium-N concentration should be able to support the culture for up to 612 h of high performance, stable operation for the influent conditions considered. To provide the additional N required for at least 720 h of continuous operation without further altering the initial ionic strength of the medium, pH control was now performed using a base mixture of 5.5 M KOH and 0.5 M NH_4OH to provide additional N throughout the course of the experiment. According to preliminary data (not shown), the rate of base addition that would be associated with the expected benzene elimination rates should provide enough additional N to support at least another 150 hours of continuous operation using this formulation. A

constant benzene loading rate which averaged 425 ± 35 mg/h was applied after inoculation of the bioscrubber. From Fig. 4a, excellent performance was observed, as indicated by an average elimination capacity of 141 ± 12 g/m^3 h at over 99% removal efficiency, which was maintained throughout the duration of the 720 h experiment. No significant benzene accumulation occurred in the liquid phases of the bioscrubber (Fig. 4b) as dissolved concentrations always remained low, however, slight fluctuations did arise and a somewhat cyclical pattern of an irregular period in the dissolved benzene concentrations was observed. The reason for this behavior is not known, but could be related to metabolic variations, fluctuations in the influent benzene load, or, could be an analytical artifact. After an initial accumulation period, a steady-state biomass concentration of 14.6 ± 0.5 g-CDW/L was achieved and maintained for over 400 h until completion of the experiment (Fig. 4c). Dissolved oxygen levels remained between 91 and 78% of saturation during the 30 day period, indicating no oxygen limitation.

Discussion

After 720 h of continuous benzene treatment at an average elimination capacity of 141 ± 12 g/m^3 h and over 99% removal efficiency, including over 400 h of operation at a steady-state biomass concentration, the ability to operate and maintain TPPB performance during a long-term application without medium exchanges has been demonstrated conclusively. Eliminating medium exchanges not only simplifies the routine operation of this process and reduces labor costs, but also reduces the potential expenses associated with materials and waste disposal. Table 2 compares the waste material generated during this study to

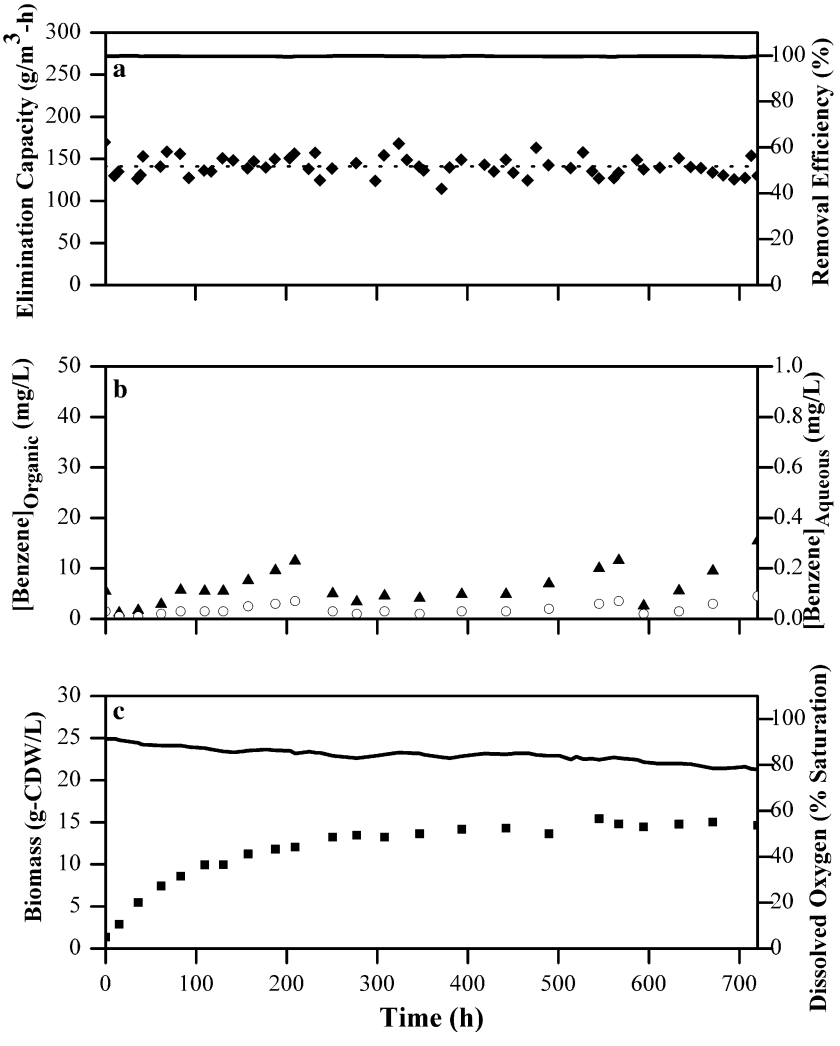

Fig. 4 Bioscrubber performance during 30 days of continuous treatment of a waste gas containing 5.6 ± 0.5 mg/L fed at 75 L/h (0.42 vvm), as represented by **a** Benzene elimination capacity (*diamonds*), average elimination capacity of 141 ± 12 g/m^3 h (*dashed line*), and removal efficiency (*solid line*), **b** organic (*triangles*) and aqueous (*circles*) phase benzene concentrations and, **c** biomass concentration (*squares*) and dissolved oxygen level (*solid line*) throughout the successful long-term treatment

that of Davidson and Daugulis [8], and also to the predicted material wastes that would be have been generated had medium exchanges been applied according to the protocol followed by Davidson and Daugulis [8] during a benzene treatment of the scale and duration studied here. Wasted biomass has little inherent value in this application, and thus expenses associated with its disposal would add to the overall operating costs. By not performing medium exchanges the total biomass produced over the 30 day treatment can be reduced nearly fivefold. Furthermore, by retaining the biomass at high concentrations, the total potential for benzene consumption in the TPPB remains high. This could prove to be a valuable attribute of this process, particularly when faced with dynamic fluctuations in the feed stream. The organic phase removed from the bioscrubber during medium exchanges could likely be recycled at high recovery rates and not altogether wasted. However, doing so would require additional equipment and handling. Finally, losses associated with wasted aqueous medium are primarily attributed to its treatment as a potentially hazardous effluent, as well as with the loss of unused inorganic nutrients.

While previous studies on benzene vapor treatment using a TPPB have also outperformed most biofilter studies in terms of high elimination capacities and removal efficiencies [7, 8], their demonstration in a long-term applications has remained to be shown. Biofiltration and trickling biofiltration remain attractive since these units routinely run in prolonged studies with benzene for several months [18], thanks largely to their undemanding operation. The present study, however, has reduced the contrast between the operating protocols of the TPPB and other vapor-phase bioreactors by demonstrating that the TPPB can be employed in long-term treatments with similar ease. Since the operating policies of the TPPB now draw a closer resemblance to more traditional vapor-phase bioreactors, a direct comparison of these technologies for the treatment of benzene contaminated waste gases is more appropriate. The data of Table 3 reflect the fact that biofilters and trickling biofilters are typically best suited to treat high volume gases with low benzene content [19], since few of the feed concentrations exceed even 1 g/m^3. In contrast, the influent benzene concentration averaged 5.6 g/m^3 for the TPPBs in this study. Furthermore, the 141 ± 12 g/m^3 h average elimination capacity achieved in this study is roughly seven times higher than the highest value reported in Table 3 for biofilters. Although the superficial gas velocities used in some previous studies are on par with those used here, it is clear that biofilters have typically been used to treat waste gases at significantly higher flow rates. Therefore, future work to investigate TPPB performance under such conditions will be invaluable to the development of this technology, particularly for assessing its practical scale-up prospects. Finally, in terms of demonstrating sustainability, only the benzene biofiltration study by Sene et al. [18] lasted longer than this experiment, though achieving much poorer performance.

During active growth stages (after start-up and during the transient) of the preliminary experiment, a large portion of the benzene-C consumed was being assimilated into biomass, and N was correspondingly

Table 2 Comparing experimental and theoretical material wastes associated with Davidson and Daugulis [8] and their medium exchange protocol with this study and its reformulated operating strategy for the long-term treatment of 141 g/m^3 h benzene using a TPPB

Study	Davidson and Daugulis [8]	Predicted scale-up and extension of Davidson and Daugulis [8]	Present study
Total liquid volume (L)	1	3	3
Duration of experiment (h)	140	720	720
Period between exchanges (h)	20	20	–
Target [biomass] (g-CDW/L)	2–4	2–4	–
Exchanges required	7	36	0
Wasted per exchange/total wasted on exchanges during treatment period			
Aqueous medium (L)	0.33/2.31	1/36	0/0
Organic phase (L)	0.17/1.19	0.5/18	0/0
Biomass (g)	1.32/9.24	4/144	0/0
Disposed at end of treatment period			
Aqueous medium (L)	0.66	2	2
Organic phase (L)	0.34	1	1
Biomass (g)	2.64	8	29
Total wasted during treatment period			
Aqueous medium (L)	2.97	38	2
Organic phase (L)	1.53	19	1
Biomass (g)	11.9	152	29

Table 3 Performance of biofilters for the removal of benzene as a sole-substrate, as reported in selected previous biofiltration studies, and compared to this study

Bioreactor/filter media	Inlet concentration (g/m^3)	Superficial gas velocity (m/h)	Loading rate (g/m^3 h)	Elimination capacity (g/m^3 h)	Removal efficiency (%)	Period of operation (days)	Reference
Biofilter/sugarcane bagasse–glass bead mixture	0.01–0.05	30.6–122.4	6.12	3.8	43	115	[18]
Biofilter/sugarcane bagasse	0.4	31–122	12	6.4	53	12–15	[20]
Biofilter/peat	0.4	31–122	31	26	84	12–15	[20]
Biofilter/granular activated carbon mixture	0.7	4–8.7	21	20.1	96	14	[21]
Biofilter/compost-wood chips	–	–	–	7–8	–	–	[22]
Trickling biofilter/fibrous bed	0.37–1.7	1.8–3.62	2.6–25.6	11.5	> 90	–	[23]
Trickling biofilter/coal particles	0.47	4.9	64	57.6	90	–	[24]
TPPB	5.6	3.3	142	141	> 99	30	This study

consumed for use in biomass production. The ratio of the respective rates of N uptake and biomass (X) production represents the weight fraction of N assimilated into the biomass, w_N:

$$w_N = \frac{dN/dt}{dX/dt} \quad (1)$$

Using this approach and measured rates of N uptake and biomass production, the weight fraction of N in *A. xylosoxidans* Y234 during initial start-up and transient operating phase respectively, were 0.20 ± 0.03 and 0.18 ± 0.04. In an analogous manner, data from the preliminary growth stage were used to predict a P weight fraction of 0.04 ± 0.01. Bailey and Ollis [25] describe a typical *Escherichia coli* cell to have weight fractions of 0.14 and 0.03 for N and P, respectively, which agree well with our findings. The rate of N uptake during steady state, maintenance mode operation was 3–6 times lower than that measured during active growth for the same benzene elimination rate. This supports the notion of a steady-state culture whose metabolic activities predominantly exist in a 'maintenance state' during which most of the cellular activity is directed towards energy generation with minimal biosynthesis. The N assimilated during this period may be used for cellular repair or could be incorporated into internal storage materials. Similar ultimate C/N ratios were measured during both experiments in which nutrient limitations were permitted to occur. The measured value of approximately 20 is considerably higher than the ratio of 5 typically recommended by Metcalf and Eddy [26] for the biological treatment of wastewaters. Use of a higher C/N ratio is possible in the steady-state TPPB because a large proportion of the consumed benzene is used to supply maintenance energy requirements, and less biosynthesis (and, therefore, less N assimilation) occurs. This knowledge regarding inorganic nutrient requirements will help lead to more effective formulation of media to meet specific operating objectives without wasteful oversupply. Furthermore, this information could be used to help develop a preemptive nutrient supplementation strategy of the types routinely practiced in biofilter applications [27–29].

This study has helped to elucidate some of the key biological factors responsible for the performance of the TPPB, as well as the essential requirements for supporting their activity. However, these results raise other important questions which would be better addressed through additional experimentation. For instance, the extent of benzene degradation may be better assessed through a carbon balance which would

include monitoring the evolution of CO_2. Likewise, performing a rigorous nitrogen balance to include examination of the elemental composition of the cells during different growth modes would help to more accurately predict inorganic nutrient requirements. Finally, the speculated identity of 2-HMS as the predominant metabolic intermediate could be further confirmed through either GC–MS or HPLC-MS analysis of the aqueous samples. These additional topics are currently being investigated.

Conclusions

This study has successfully confirmed the potential of the TPPB for benzene treatment in a long-term application lasting 30 days. By demonstrating that nutrient requirements can be readily predicted, that potentially detrimental metabolic intermediates do not accumulate in the medium, and that cellular viability can be maintained over long periods in a closed liquid system, the need for medium exchange has been eliminated without compromising the performance or stability of the process. By translating the notion of 'maintenance state' operation into an effective and simplified operating protocol, benefits such as significantly reduced material and operating costs become immediately apparent. This less labor-intensive approach allows the operation of the TPPB to more closely resemble that of other vapor-phase bioreactors. Meanwhile, the high benzene elimination capacities and removal efficiencies demonstrated make the TPPB an attractive option among emerging biological technologies for the treatment of benzene waste gases.

Acknowledgments The financial support of the Natural Sciences and Engineering Research Council of Canada and Queen's University, in the form of research grants and a graduate scholarship, is gratefully acknowledged. Special thanks to the Analytical Services Unit at Queen's University for their assistance with ICP-AES analysis.

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