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ORIGINAL PAPER

Direct estimation of the oxygen requirements of *Achromobacter xylosoxidans* for aerobic degradation of monoaromatic hydrocarbons (BTEX) in a bioscrubber

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Abstract The O_2 requirements for biomass production and supplying maintenance energy demands during the degradation of both benzene and ethylbenzene by *Achromobacter xylosoxidans* Y234 were measured using a newly proposed technique involving a bioscrubber. Using this approach, relevant microbial parameter estimates were directly and simultaneously obtained via linear regression of pseudo steady-state data. For benzene and ethylbenzene, the biomass yield on O_2, Y_{X/O_2}, was estimated on a cell dry weight (CDW) basis as 1.96 ± 0.25 mg CDW mgO_2^{-1} and 0.98 ± 0.17 mg CDW mgO_2^{-1}, while the specific rate of O_2 consumption for maintenance, m_{O_2}, was estimated as 0.041 ± 0.008 mgO_2 mg CDW^{-1} h^{-1} and 0.053 ± 0.022 mgO_2 mg CDW^{-1} h^{-1}, respectively.

Keywords Bioscrubber · BTEX · Maintenance energy · Oxygen requirements

Introduction

The capacity of microorganisms to degrade volatile organic compounds (VOCs) aerobically has led to the development of numerous biologically based technologies for their control and treatment from wastewaters and, in particular, waste gases. Because dissolved O_2 limitations can often hinder the performance of biological treatment processes, reliable prediction of the O_2 requirements of a culture is essential to ensure that sufficient O_2 can be provided to meet the biological demand. Common means of predicting O_2 requirements include the use of theoretical relationships, such as those developed by McCarty (1965), Erickson et al. (1978) and Heijnen and Roels (1981). However, in some cases, these relationships may rely upon inappropriate assumptions regarding the nature of the biotransformation or extent of the conversion. For instance, traditional theoretical relationships are most suitable for compounds that are completely mineralized through oxidation reactions and less appropriate if the specific multistep biodegradation pathway involves preliminary oxygenation reactions, as with benzene (Yuan and Van Briesen, 2002). Alternatively, O_2 requirements can be experimentally obtained through a material balance using aerobic batch data. However, when dealing with VOC substrates, the stripping losses incurred as a result of aeration must be accounted for thereby necessitating the collection of off-gas concentration data and complicating the calculations. Furthermore, O_2 requirements measured during a batch process will be primarily growth-associated because

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specific growth rates, and corresponding substrate and O_2 consumption rates, remain high throughout the majority of the experiment. These conditions provide little opportunity to investigate the O_2 requirements associated with maintenance activities, which are best observed at lower specific growth rates. When predicting microbial O_2 requirements associated with VOC degradation, a simple alternative is to continuously supply substrate as a vapour by simply modifying the bioreactor to operate in the manner of a bioscrubber. Bioscrubbers have previously been demonstrated as effective processes for quantifying the growth-associated and maintenance energy requirements of VOC substrates (Nielsen et al. 2005a), and that notion is extended and translated here for the analogous study of associated O2 requirements.

BTEX compounds (benzene, toluene, ethylbenzene, and xylenes) represent an important class of VOCs whose biodegradation rates are commonly studied in bioreactor systems as liquid- and vapour-phase contaminants alike. Rates of benzene and ethylbenzene consumption by *A. xylosoxidans* Y234 as sole-substrates originating in simulated waste gas streams were previously studied in an aqueous-organic partitioning bioscrubber (Nielsen et al. 2005a). Following absorption into the bioscrubber, the consumed substrates were allocated between growth and maintenance activities as a function of the specific growth rate, μ, in a manner originally described by Pirt (1965). As such, the total volumetric rate of substrate utilization, r_S, can be expressed as:

$$r_S = \frac{1}{Y_{X/S}} r_X + m_S X \tag{1}$$

where $Y_{X/S}$ is the biomass yield on the substrate, m_S is the specific rate of substrate consumption for maintenance, and X is the concentration of biomass. The total volumetric growth rate of the culture, r_X, is defined by:

$$r_X = \mu X = \frac{dX}{dt} \tag{2}$$

However, just as aerobic heterotrophic microbes must metabolize organic substrates to satisfy their growth and maintenance energy requirements, so too must they consume O_2 through respiration to perform both of these functions. Therefore, the total rates of substrate and O_2 consumption are directly related, each depending upon the metabolic state of the culture (i.e., distribution between growth and maintenance) and the specific growth rate of the organism. The total volumetric rate of O_2 consumption, r_{O_2}, can then be analogously described as:

$$r_{O_2} = \frac{1}{Y_{X/O_2}} r_X + m_{O_2} X \tag{3}$$

where Y_{X/O_2} is the biomass yield on the O_2, and m_{O_2} is the specific rate of O_2 consumption for maintenance purposes. Normalization of Eq. 3 with respect to the biomass concentration, X, yields the following expression:

$$\frac{r_{O_2}}{X} = \frac{1}{Y_{X/O_2}} \mu + m_{O_2} \tag{4}$$

which can also be expressed as:

$$Q_{O_2,TOT} = Q_{O_2,G} + Q_{O_2,M} \tag{5}$$

Equation 5 states that the total specific rate of O_2 consumption, $Q_{O_2,TOT}$, is composed of the specific rates of O_2 consumption for growth and maintenance, $Q_{O_2,G}$ and $Q_{O_2,M}$, respectively. This result is analogous to the linear relationships developed by Pirt (1965) for substrate utilization.

In a previously published study of the response of an aqueous-organic partitioning bioscrubber to fluctuating benzene feeds it was observed that during brief (4 h) transient periods while a constant loading rate of benzene was fed to the bioscrubber (and treated at a high removal efficiency), biomass slowly accumulated in response to the availability of additional substrate. Since the process was always limited by the benzene absorption rate (under the conditions examined), observed increases in biomass always remained linear and substrate was never available in such an excess that exponential growth could be supported. As such, during the brief transient periods the rate of change of biomass remained effectively constant, meaning instantaneous estimates of the specific growth rate could be easily obtained at any time

according to its definition ($\mu = (dX/dt)/X$) using direct measures of both dX/dt and X. Furthermore, during these transitory periods, a constant dissolved O_2 concentration, $[O_2]$, was also achieved in the aqueous phase and maintained throughout (Nielsen et al. 2005b). The specific constant value of $[O_2]$ achieved during each transient was dependent upon the benzene-loading rate applied as well as the mass of cells present. This was because, while the particular loading rate applied influenced the growth-associated O_2 consumption, the total mass of cells affected the overall maintenance rate and the two terms are additive according to Eq. 5.

A constant dissolved O_2 concentration implies that the rate of O_2 transfer to the aqueous phase is equivalent to the rate at which it is being utilized by the cells, or:

$$r_{O_2} = k_L a([O_2]^* - [O_2]) \quad (6)$$

where $[O_2]^*$ represents the saturation concentration of dissolved O_2 in the aqueous medium and $k_L a$ is the volumetric mass transfer coefficient. The total volumetric rate of O_2 consumption, r_{O_2}, is therefore easily measured under these pseudo steady-state conditions using either experimental data or theoretical correlations to estimate $k_L a$ and $[O_2]^*$ for the specific set of operating conditions considered. Therefore, according to the relationship of Eq. 4 (and definition of Eq. 5), the quantities Y_{X/O_2} and m_{O_2} are easily estimable by linear least-squares regression of $Q_{O_2,TOT}$ vs. μ. This proposed approach of directly and simultaneously estimating characteristic parameters Y_{X/O_2} and m_{O_2} is now applied to the aerobic degradation of benzene and ethylbenzene in a bioscrubber in order to demonstrate its capabilities.

Materials and methods

Organism, media, and cultivation

Achromobacter xylosoxidans Y234, a known BTEX degrader, was cultivated and prepared for inoculation in the bioscrubber using methods and media that have been previously described (Nielsen et al. 2005a).

Bioscrubber configuration

All experiments were conducted using a 6 l New Brunswick Scientific BioFlo III bioreactor containing 3 l of aqueous medium with the following composition: 14 g l^{-1} $(NH_4)_2SO_4$, 1.5 g l^{-1} $MgSO_4 \cdot 7H_2O$, 13.2 g l^{-1} K_2HPO_4, 16.8 g l^{-1} KH_2PO_4, and 2 ml l^{-1} trace elements solution (16.2 g l^{-1} $FeCl_3 \cdot 6H_2O$, 9.44 g l^{-1} $CaHPO_4$, 0.15 g l^{-1} $CuSO_4 \cdot 5H_2O$, and 40 g l^{-1} citric acid). Although the focus of our previous research has predominantly been on the removal and treatment BTEX waste gases using an aqueous-organic partitioning bioscrubber, only a single aqueous phase is employed here with the objective of simplifying the material balance equations. The bioscrubber was controlled and operated at the nominal conditions of pH 6.6, 30°C, and agitation at 800 rpm. The gaseous feed contained dilute concentrations of benzene or ethylbenzene substrate, generated as previously described (Nielsen et al. 2005a), was sparged into the bioscrubber at 90 l h^{-1} for benzene experiments and 60 l h^{-1} for ethylbenzene experiments. Feed-gas concentrations were changed by adjusting the relative flows of BTEX-rich and make-up air streams.

Sampling and analysis

Benzene and ethylbenzene content in the feed- and off-gas, as well as the liquid phase, were determined by GC-FID using methods previously described (Nielsen et al. 2005a). Biomass concentrations were quantified using optical density measurements and compared to cell dry weight (CWD) calibrations as previously described (Nielsen et al. 2005a). Dissolved O_2 concentration was measured with a polarographic membrane dissolved O_2 probe and recorded using a computer interface at 5 min intervals.

Results and discussion

The proposed approach of estimating Y_{X/O_2} and m_{O_2} was first applied using a continuous bioscrubber into which a gas was fed at 90 l h^{-1}, containing an average benzene concentration of

3.1 ± 0.3 mg benzene l^{-1}, corresponding to an initial loading rate of 278 ± 27 mg benzene h^{-1}. Throughout the duration of this initial loading, off-gas benzene concentrations remained below 0.04 mg benzene l^{-1}, resulting in high average removal efficiencies and elimination capacities of 99.1 ± 0.8% and 92 ± 9 mg benzene l^{-1} h^{-1}, respectively. Benzene accumulation in the aqueous phase was not observed at any time during the loading period, with dissolved levels never exceeding 0.1 mg benzene l^{-1}, indicating that the overall process was mass transfer limited. As benzene was consumed in the first stage of the experiment, the dissolved O_2 level stabilized at about an average level of 92.1 ± 0.7% of saturation, as shown in Fig. 1.

After 10.7 h, the average loading rate was increased to 912 ± 49 mg benzene h^{-1} by increasing the average feed gas benzene concentration to 9.9 ± 0.6 mg benzene l^{-1}. Throughout the second loading stage, removal efficiencies remained high at 98.7 ± 0.6% and the elimination capacity averaged 300 ± 7 mg benzene l^{-1} h^{-1}. Again, off-gas and aqueous benzene concentrations remained relatively low and constant, stabilizing at below 0.2 mg benzene l^{-1} and 0.5 mg benzene l^{-1}, respectively. The cells responded quickly to the additional substrate supply, producing additional biomass, minimizing aqueous accumulation and consuming additional dissolved O_2. As seen in Fig. 1, dissolved O_2 levels approach a new steady value of 76.8 ± 0.3 % approximately 3 h after the loading step change was performed. Due to the brief period of each loading rate studied, the corresponding volumetric growth rate of cells (equivalent to the slope of X vs. time) obtained at each level approaches a linear increase, indicating that the culture is limited by the rate of benzene supply by absorption.

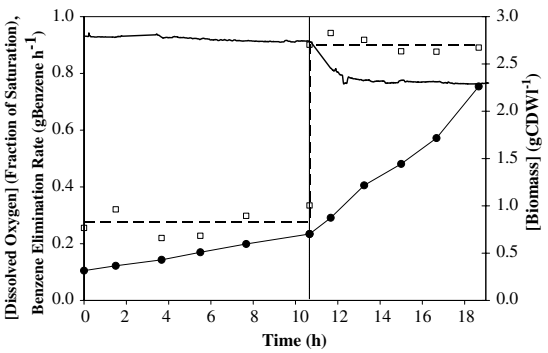

Fig. 1 The response of the biomass concentration (solid circles) and dissolved O_2 level (solid line) to a step input (initiated, as shown by the dotted line, after 10.7 h) in the benzene elimination rate (open squares). Average benzene elimination rates at each step are also shown (dashed line)

The correlation of Nielsen et al. (2005c) was used to estimate $k_L a$ as 85.2 h^{-1} in this 3 l aqueous system agitated at 800 rpm and aerated at 90 l h^{-1}. $Y_{X/S}$ and m_S were set to average values of 0.69 mg CDW mg benzene^{-1} and 0.0173 mg benzene mg CDW^{-1} h^{-1}, respectively, using the data of Nielsen et al. (2005a). $[O_2]^*$ was estimated as 7.9 mgO_2 l^{-1}, the concentration of dissolved O_2 in pure water in equilibrium with air at 1 atm and 30°C (Atkins, 1994). Parameter estimation was conducted according to the relationship of Eq. 4 using data from both loading stages but only from those regions where the dissolved O_2 concentration had stabilized at a pseudo-steady-state value. X values were measured at each sampling point and used to calculate $Q_{O_2,TOT}$ and μ. The results are shown in Fig. 2. Linear least-squares regression of the benzene degradation data yielded estimates of Y_{X/O_2} of 1.96 ± 0.25 mg CDW mgO_2^{-1} from the reciprocal of the estimated slope, and m_{O_2} of 0.041 ± 0.008 mg O_2 mg CDW^{-1} h^{-1} from the intercept.

The experiment was then repeated using ethylbenzene as the sole substrate, the results of

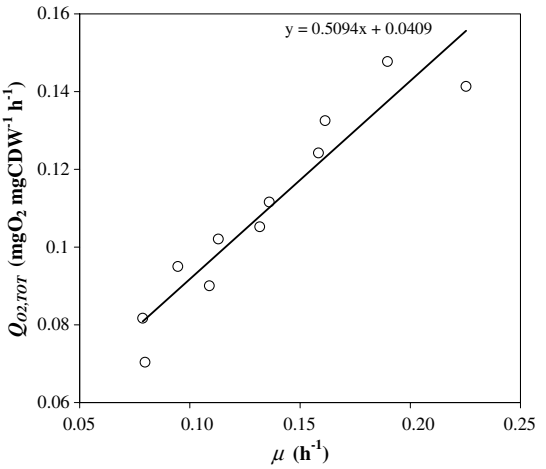

Fig. 2 Estimation of Y_{X/O_2} and m_{O_2} via linear regression for benzene degradation using the relationship described by Eq. 4

which will be discussed only briefly since the phenomenological behaviour was essentially identical to that of the benzene experiment. Ethylbenzene was initially fed to the bioscrubber at a concentration of 1.8 ± 0.2 mg ethylbenzene l^{-1} at 60 l h^{-1}, comprising an initial loading rate of 103 ± 9 mg ethylbenzene h^{-1}. Removal efficiencies and elimination capacities were maintained above 91% and 31.2 ± 2.7 mg ethylbenzene l^{-1} h^{-1}, respectively, for 4 h while dissolved O_2 levels remained at approximately 96% of saturation and biomass levels slowly increased. The loading rate was then increased to 201 ± 15 mg ethylbenzene h^{-1} by increasing the feed concentration to 3.4 ± 0.2 mg ethylbenzene l^{-1}. At the second loading rate, a removal efficiency of over 92% was maintained for an additional 4.25 h while achieving an average elimination capacity of 62.2 ± 4.5 mg ethylbenzene l^{-1} h^{-1}. Biomass accumulated more rapidly during the second loading period and dissolved O_2 concentrations achieved a new steady state of about 93% roughly 2.5 h after the step change. The proposed estimation method was repeated for ethylbenzene, however using an estimate of k_La of only 71.1 h^{-1} at 60 l h^{-1} and 800 rpm (Nielsen et al. 2005c). $Y_{X/S}$ and m_S were also set to 1.1 mg CDW mg ethylbenzene^{-1} and 0.017 mg ethylbenzene mg CDW^{-1} h^{-1}, respectively, according to the data of Nielsen et al. (2005a). Equation 4 was applied to the appropriate data and plotted in Fig. 3 to the obtain parameter estimates associated with ethylbenzene degradation. Using linear least-squares regression, the parameter estimates found for ethylbenzene degradation were Y_{X/O_2} of 0.98 ± 0.17 mg CDW mgO_2^{-1} and m_{O_2} of 0.053 ± 0.022 mgO_2 mg CDW^{-1} h^{-1}.

The substrate consumed for maintenance energy purposes should be completely oxidized in order to release the greatest amount of energy to the cells. Since the complete oxidation of benzene requires 3.07 mg O_2 mg benzene^{-1}, it implies that for benzene:

$$m_{O_2} = 3.07 m_S \quad (7)$$

or, that m_{O_2} for the growth of *A. xylosoxidans* Y234 on benzene should be on the order of 0.053 ± 0.001 mg O_2 mg CDW^{-1} h^{-1}. Likewise,

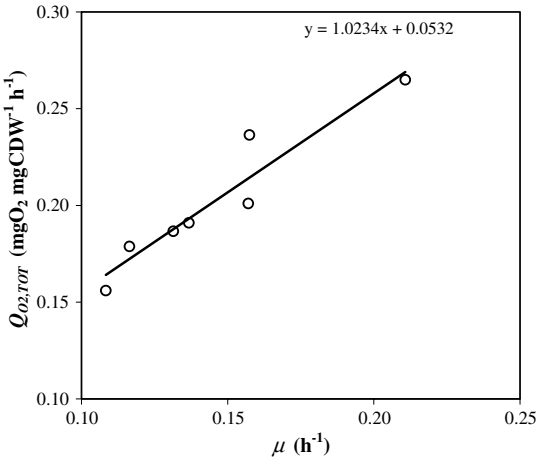

Fig. 3 Estimation of Y_{X/O_2} and m_{O_2} via linear regression for ethylbenzene degradation using the relationship described by Eq. 4

complete oxidation of ethylbenzene would require 3.17 mgO_2 mg ethylbenzene^{-1}, meaning that m_{O_2} would theoretically be 0.054 mg O_2 mg CDW^{-1} h^{-1}. This assumption and the approximated values are in close agreement with the derivations of Heijnen and Roels (1981). The estimates of m_{O_2} of this organism for benzene and ethylbenzene, as well as their respective theoretical values, are numerically similar because the degrees of reduction of benzene and ethylbenzene are so alike. This means that a comparable number of electrons are released during the oxidation of each compound (Shuler and Kargi 1992). The methods of Heijnen and Roels (1981) have also been used to estimate theoretical maxima of Y_{X/O_2} for the growth of a typically composed bacterium on benzene and ethylbenzene as 2.83 mg CDW mgO_2^{-1} and 2.26 mg CDW mg O_2^{-1}, respectively, under which both of our estimates fall. Furthermore, as theoretically predicted, Y_{X/O_2} is significantly greater for the degradation of benzene than ethylbenzene.

In the two examples discussed, degradation rates sufficiently exceeded VOC absorption rates such that the process was always mass transfer limited and no significant substrate accumulation occurred in the liquid. This condition is preferred for the purposes of the proposed method because it leads to the simplified material balances derived

in Equations 1 and 3, as well as linear biomass growth. Therefore, substrate-loading rates should be selected to accommodate this simplifying condition. It was assumed for this study that the nutrient medium will have the same equilibrated dissolved O_2 properties as pure water, however, it is well-known that dissolved salts can affect the solubility of O_2 in an aqueous solution (Shuler and Kargi 1992). Since the predicted value of r_{O_2} will be particularly sensitive to the values of $[O_2]^*$ and $[O_2]$, this assumption will affect the numerical values of the predicted parameters, particularly for concentrated solutions. Likewise, since the proposed relationship involves the use of several design and operational parameters, accurate numerical results will ultimately depend upon the adequate measurement of each of them. However no such deviations will affect the linear relationship described.

Conclusion

The O_2 requirements of *Achromobacter xylosoxidans* Y234, represented through the biomass yield and maintenance energy parameters Y_{X/O_2} and m_{O_2}, respectively, for the consumption of both benzene and ethylbenzene as sole substrates were directly quantified using pseudo steady-state dissolved O_2 data obtained in a bioscrubber. Although the presented estimation technique has thus far only seen limited application, it is anticipated that this straightforward method will be well-suited for use with various other VOCs, and further validation will help to confirm its applicability.

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