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ORIGINAL PAPER

Estimating the cellular maintenance coefficient and its use in the design of two-phase partitioning bioscrubbers

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Abstract One of the key roles of an organic solvent has emerged to be the enhancement of oxygen transfer in two-phase partitioning bioscrubbers (TPPBs). In order to determine an optimum organic fraction for a given VOCs loading, the oxygen demand of the total cell mass must be estimated, which depends upon the magnitude of the cellular maintenance coefficient. We have estimated the dynamics of the maintenance coefficient for benzene degradation by *Achromobacter xylosoxidans* Y234 in a TPPB and found that the maintenance coefficient generally decreased as cells accumulated in the TPPB but converged to a specific value of 1.750×10^{-2} h^{-1} at biological steady state. Due to its important influence on all of the essential design parameters of the TPPB system, including optimum organic fraction, aeration rate and agitation speed, the maintenance coefficient should be considered as a key biological determinant for microorganism selection, as well as in overall TPPB design.

Keywords Two-phase partitioning bioscrubber · Benzene · Maintenance coefficient · Cell mass · Loading rate · Oxygen requirement

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Introduction

Of the biologically based technologies presently available for the treatment of VOCs, biofiltration has been shown to be effective for the treatment of low concentrations of VOCs [1–3]. As an alternative technology, the two-phase partitioning bioreactor (TPPB) has been effectively applied to the treatment high concentrations of pollutants [4, 5]. The first version of the TPPB that was successfully applied to VOCs consisted of a two-stage unit [6] and required considerable operator intervention to maintain the two phases distinct, and to operate the system at steady state. These difficulties led to the design of a two-phase partitioning bioscrubber (TPPB) in which aqueous and organic phases were completely mixed as a single homogenous phase [7, 8]. We have recently shown the potential of replacing the liquid organic phase of TPPBs with inexpensive polymers as a second phase, which has the advantage of allowing the use of microbial consortia, while also eliminating operational challenges (e.g., emulsions) which sometimes arise in two-liquid phase TPPBs [9].

Recently, many other researchers have begun to apply the concept of the TPPB to the treatment of various VOCs and other contaminants [10–15]. Although this research has generally been successful, encouraging TPPBs to be applied to other target molecules, the approach has largely been heuristic with no general strategies being proposed to aid in, for example, solvent selection, specification of organic phase fraction, operating conditions, etc. We have recently generated a set of heuristic criteria and proposed a guideline for the development of TPPBs [8] in which we first addressed the necessity of employing a TPPB based on physical considerations of mass transfer (VOCs and oxygen), organic solvent properties and pollutant properties (e.g., Henry's Law constant). In this study, we now focus

on the biology associated with these systems and suggest a general method with which to calculate cellular maintenance coefficients as well as cell mass requirements for various inlet VOC loading rates. Although the calculations of maintenance coefficient have been performed by various methods [16–20], the basic concepts were based on a material balance accounting for cell growth and maintenance, and the assumption that the maintenance coefficient was constant. In this study, a general material balance for the TPPB, a unique continuous bioreactor system with cells being retained, was again used for the calculation of maintenance coefficient and this estimate implicated the possibility of dynamic behaviour of the maintenance coefficient. Finally, we discussed the role of maintenance coefficients in TPPB design and operation.

Materials and methods

Experimental conditions

The experimental data utilised in this paper are based on the degradation of benzene by *Achromobacter xylosoxidans* Y234 in a TPPB with hexadecane as the sequestering phase, and come from the work of Nielsen et al. [20]. The experimental conditions including microorganism, culture medium, operation condition, configuration of TPPB, etc. were described in the same paper.

Theoretical background: maintenance coefficient

For more than 50 years, the concept of cellular maintenance has been understood in two ways. Endogenous metabolism, later termed the specific maintenance rate, is referred to as a decay or negative relative growth rate in which the sum of specific maintenance rate multiplied by microbial cell mass equals the total rate at which biomass is lost through maintenance [21, 22]. Maintenance supply, later termed maintenance coefficient m by Pirt [17], is an alternative concept which defines the minimum rate of substrate consumption for maintaining cells. In the latter approach, maintenance denotes an additional substrate consumption which is not used for growth purposes [18, 20, 23, 24]. The concept of the maintenance requirement as negative growth or endogenous metabolism seems to be artificial and indirect [17]. The term maintenance coefficient has been preferred possibly because it can be easily calculated with observed cell growth yield and specific growth rate and may have more physiological meaning [17, 25]. In addition, the specific growth rate in this definition represents not the "true" specific growth rate as it does in the specific maintenance rate but "net" relative specific growth rate, which makes it easy to measure this value in actual experiments. For these reasons, we have chosen to use the maintenance coefficient concept for the purposes of this study.

For the present analysis, we have adopted a set of four basic assumptions for TPPB operation, on the basis of the study by Nielsen et al. [20]:

1. Cells exist only in the aqueous phase.
2. The liquid phase comprised of aqueous and organic phases is completed mixed.
3. Benzene is the only limiting factor for cell growth.
4. Gas flow rate is constant through the TPPB.

With these assumptions, the material balance equation for benzene in the liquid phases of the TPPB can be expressed with respect to physical and biological terms as:

$$FC_{\mathrm{i}} - FC_{\mathrm{f}} - \frac{1}{Y_{\mathrm{x/s}}}\mu V_{\mathrm{A}} X_{\mathrm{A}} - mV_{\mathrm{A}} X_{\mathrm{A}} = V_{\mathrm{O}}\frac{\mathrm{d}C_{\mathrm{O}}}{\mathrm{d}t} + V_{\mathrm{A}}\frac{\mathrm{d}C_{\mathrm{A}}}{\mathrm{d}t} \quad (1)$$

where, F, C_{i} and C_{f} represent gas flow rate (L/h), inlet and outlet gaseous benzene concentrations (mg/L), respectively. X_{A}, $Y_{\mathrm{x/s}}$, μ and m are cell concentration in the aqueous phase (mg/L), cell growth yield on benzene (mg cell/mg benzene), specific growth rate (1/h) and maintenance coefficient (mg-benzene/mg cell/h), respectively. In particular, it should be noted that $Y_{\mathrm{x/s}}$ is not the "apparent or observed cell growth yield" but the "true cell growth yield" which, per its definition, accounts for substrate consumed directly for growth purposes only. V_{O}, V_{A}, C_{O}, C_{A} and t are the volumes of organic and aqueous phases (L), benzene concentrations in the organic and aqueous phases (mg/L), and time (h), respectively.

We considered four different scenarios influencing Eq. 1 depending on the cell growth or accumulation of benzene in the organic phase of the TPPB, as depicted in Fig. 1. The cells need time to induce relevant enzymes to degrade benzene, during the adaptation period (Period 1) and benzene accumulates in the TPPB during this period. After adaptation, the cells start to degrade benzene vigorously with cell concentration consistently increasing, and benzene concentration decreasing (Period 2). If the TPPB is operated in a mode that remains mass transfer limited (as is most typically the case), the benzene concentration in the organic phase will rapidly decrease during the 2nd period as shown by the dashed line instead of the solid one in Fig. 1. Thereafter, cell growth slows due to increasing consumption of benzene for maintenance by high cell concentrations and thus low benzene concentration in the aqueous phase (Period 3). Finally, all the benzene is used for cell maintenance only and no cell growth is observed (Period 4). Each period was subsequently expressed below as a simplified form of Eq. 1. Since instantaneous equilibrium in the TPPB can be assumed, the benzene

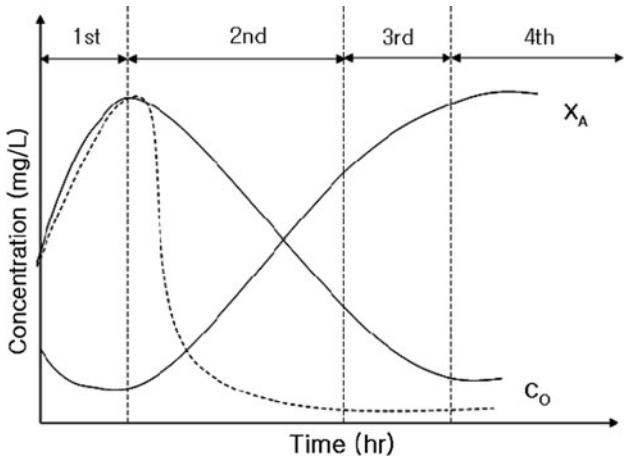

Fig. 1 Schematic diagram of biodegradation of benzene in a TTPB. X_A and C_O represent cell mass in aqueous phase and benzene concentration in organic phase, respectively

concentration in the organic phase can be expressed as a function of the aqueous phase benzene concentration by use of an equilibrium partitioning coefficient for benzene between the two phases, $P_{O\text{–}A,}$ [8].

(Period 1) No cell growth but noticeable increase of benzene concentration in organic phase

This period applies to the cell adaptation stage when cells are not yet able to degrade benzene but the organic phase continuously absorbs inlet benzene [6]. Although the cell concentration typically decreases slightly during this period, the decrease was found to be negligible possibly because of the relatively short adaptation time required ($\sim$10–15 h) and the low concentration of benzene in the aqueous phase despite benzene accumulation, which will be discussed below. No consumption of benzene by cells also can be assumed during this period. Therefore, the specific growth rate and maintenance coefficients are zero and Eq. 1 can be reduced to the following equation.

$$FC_i - FC_f = V_O\frac{dC_O}{dt} + V_A\frac{dC_A}{dt} \tag{2}$$

If we then set $C_O = P_{O\text{–}A} \times C_A$, we can obtain the equation for the benzene in the organic phase as follows:

$$\frac{dC_O}{dt} = \frac{F(C_i - C_f)}{(V_O + \frac{V_A}{P_{O\text{–}A}})} \tag{3}$$

The solution of Eq. 3 representing physical absorption of benzene will describe the profile of benzene concentration in the organic phase throughout Period 1. The unknown outlet gaseous benzene concentration, C_f, can be approximately calculated with Eq. 4 as suggested previously [26] with the assumption of rapid equilibrium between inlet gas and liquid phase, and rapid partitioning between aqueous and organic phases.

$$C_f = C_i - k_La_A(C_A^* - C_A)\frac{V_A}{F} - k_La_O(C_O^* - C_O)\frac{V_O}{F} \tag{4}$$

where, k_La_A and k_La_O are mass transfer coefficients of gaseous benzene into aqueous and organic phases, respectively and their values were given in the same reference. In addition, C_A^* and C_O^* are equilibrated benzene concentrations in the aqueous and organic phases with a given inlet gaseous benzene concentration, respectively. Finally, C_A can be readily converted to C_O with the partitioning coefficient. When using all of the available values specified for the TPPB to which 5.5 mg/L of benzene with 60 L/h of gas flow was fed, we can get the following relationship.

$$C_f \approx 2.21 \times 10^{-3} C_O \tag{5}$$

(Period 2) Substantial changes in both cell growth in aqueous phase and benzene concentration in organic phase

Since the benzene concentration in the aqueous phase was very low throughout the experimental work (phases 2–4), ranging from $\sim$0.004 to 0.119 mg/L, and the rate of change of this concentration is assumed to be negligible, the last term in Eq. 1 can be set to zero, resulting in Eq. 6.

$$FC_i - FC_f - \frac{1}{Y_{x/s}}\mu V_A X_A - mV_A X_A = V_O\frac{dC_O}{dt} \tag{6}$$

This equation can be rearranged to calculate the maintenance coefficient as follows:

$$\begin{aligned} m &= \frac{FC_i - FC_f - V_O\frac{dC_O}{dt} - \frac{1}{Y_{x/s}}\mu V_A X_A}{V_A X_A} \\ &= \frac{F(C_i - C_f) - V_O\frac{dC_O}{dt}}{V_A X_A} - \frac{1}{Y_{x/s}}\mu \end{aligned} \tag{7}$$

(Period 3) Slowing cell growth and minor change of benzene concentration in the organic phase

This period lies between vigorous cell growth and biological steady state, however, it is very difficult to distinctly divide Periods 2 and 3. Since the cells are still growing, Eq. 7 remains valid during this period.

Whenever, the change of benzene concentration in the organic phase is very small as observed in this study (see Fig. 2), benzene accumulation in the organic phase is approximately zero, resulting in Eq. 8.

$$m = \frac{FC_i - FC_f - \frac{1}{Y_{x/s}}\mu V_A X_A}{V_A X_A} = \frac{F(C_i - C_f)}{V_A X_A} - \frac{1}{Y_{x/s}}\mu \tag{8}$$

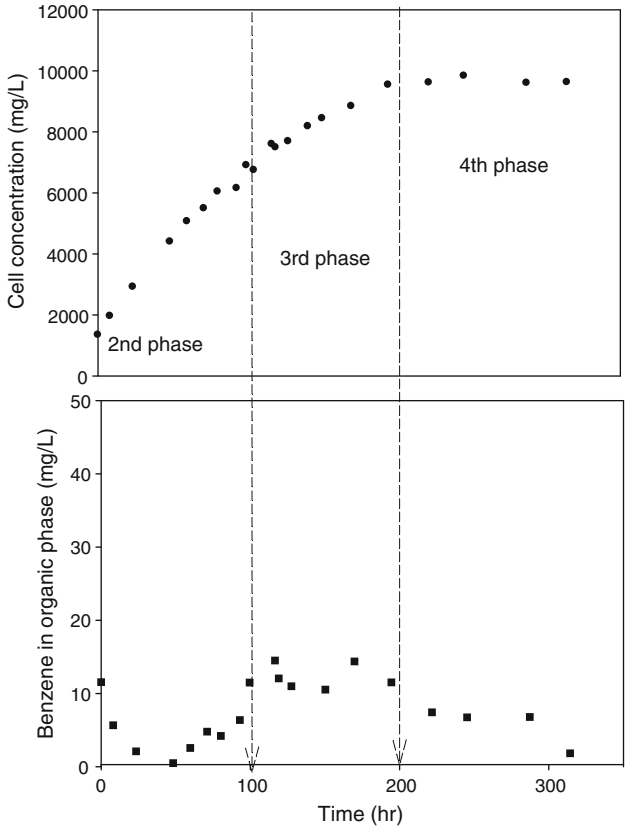

Fig. 2 Profiles of cell mass in aqueous phase and benzene concentration in organic phase in the TPPB composed of 2 L aqueous and 1 L organic phases operating at 30 °C and pH of 6.6. Average inlet benzene concentration was 5.5 mg/L with 60 L/h of gas flow rate

(Period 4) No cell growth and no change of benzene concentration in the organic phase

This period applies to biological steady state in the TPPB wherein there is no net cell growth and negligible changes to the aqueous and organic benzene concentrations. Under these conditions Eq. 1 becomes:

$$FC_{\mathrm{i}} - FC_{\mathrm{f}} - mV_{\mathrm{A}}X_{\mathrm{A}} = 0 \tag{9}$$

and, this equation can be rearranged to Eq. 10.

$$m = \frac{FC_{\mathrm{i}} - FC_{\mathrm{f}}}{V_{\mathrm{A}}X_{\mathrm{A}}} \tag{10}$$

Results and discussion

Simplifying mass balance equation

Figure 2 shows cell growth in the aqueous phase and benzene accumulation in the organic phase through Periods 2, 3, and 4. It should be noted that in the work of Nielsen et al. [20], the first sample was taken after 24 h of operation. Considering Period 1 to be 10–12 h in this system [26], all the Period 1 and approximately 13 h of Period 2 were missed in Fig. 2. The profiles of benzene concentration in the organic phase during Period 1 can be estimated using Eqs. 3 and 5. The benzene concentration in the organic phase was estimated to be 1,898.2 mg/L after 11 h of operation and this corresponds to 13.5 mg/L of benzene in the aqueous phase. Because 3,630 mg of benzene was fed to the TPPB during Period 1, 1704.8 mg of benzene was stripped out of the TPPB and 53.0% of benzene was captured in the TPPB. The sharp decrease in benzene concentration in the organic phase to around 10 mg/L and increase of cell mass from 0.1 to 1.34 g/L during the missing part of Period 2 confirm that this system follows the behaviour depicted as a dashed line in Fig. 1, implying mass transfer limitation.

Cell growth shows typical behaviour for Periods 2–4 with Period 2 ranging from about 0 to 100 h, Period 3, 100 to ~200 h and Period 4, from ~200 to 300 h, when the experiment was halted. The observed linear increase in cell mass that occurs during Period 2 again indicates that cell growth and benzene biodegradation rates are limited by the rate of benzene mass transfer [20]. Since the benzene concentration in the aqueous phase was extremely low, in the range of ~0.004–0.119 mg/L, its concentration was omitted in Fig. 2, as well as in the all calculations performed here. The benzene concentration in the organic phase was as low as ~0.5–14.4 mg/L and its profile is shown in Fig. 2. The very low concentrations of benzene in both aqueous and organic phases that exist beginning early in Period 2 suggest mass transfer limiting conditions. Observed fluctuations of the benzene concentration in the organic phase may be attributed to fluctuations in inlet feed conditions which were routinely encountered during the course of these experiments. Due to the low concentration of benzene in the organic phase, its contribution to the calculation of maintenance coefficient turned out to be negligible, which will be discussed later.

To calculate the maintenance coefficient by Eq. 7, we must first estimate profiles of the specific growth rate, μ, and $\frac{\mathrm{d}C_{\mathrm{O}}}{\mathrm{d}t}$ from the raw experimental data. The specific growth rate was defined as follows:

$$\mu = \frac{1}{X_{\mathrm{A}}}\frac{\mathrm{d}X_A}{\mathrm{d}t} = \frac{\mathrm{d}\ln X_{\mathrm{A}}}{\mathrm{d}t} \tag{11}$$

The logarithmic cell concentration was correlated with time as an exponential function of time with R^2 being 0.995 and this is shown as Eq. 12.

$$\ln X = 7.2869 + 1.9023(1 - \mathrm{e}^{-0.0171t}) \tag{12}$$

The specific growth rate may then be calculated as follows:

$$\mu = \frac{\mathrm{d}\ln X_{\mathrm{A}}}{\mathrm{d}t} = 3.25 \times 10^{-2}\mathrm{e}^{-0.0171t} \tag{13}$$

This equation indicates that the highest specific growth rate is 0.0325 h^{-1} at time zero and it decreases with time (or increasing cell mass). Through Periods 2 and 3, the specific growth rate remains in the range of 1.06×10^{-3}–3.25×10^{-2} h^{-1}, which is remarkably lower than the maximum specific grow rate of this organism on benzene, previously measured to be 0.58 h^{-1} [27]. Because specific growth rate is known to be strongly dependent on the concentration of the limiting substrate, these low values can be correlated with the low benzene concentrations in the aqueous phase.

Although the inlet benzene concentration utilised in TPPB studies are generally much higher than in those using a biofilter, (typically just 1–2 mg/L), it remains too low or alternatively, the biomass concentration is too high, for the cells to achieve their maximum specific growth rate. With μ_{max} and $Y_{x/s,obs}$ being 0.58 h^{-1} and 0.46, respectively [27], for example, from 20 to 21 h of operation, the amount of benzene required for maximum cell growth was calculated to be 2,076 mg/L or 4,152 mg. Therefore, the inlet benzene concentration of 5.5 mg/L or 324 mg/h of loading rate was too low to support high specific growth rate, and benzene in the TPPB was easily consumed. Accordingly, the benzene concentration in the organic phase approaches zero and stays at a low level in the range 0.5–14.4 mg/L and $V_O\frac{dC_O}{dt}$ varied in the range −0.7416 to 0.1930 mg/h throughout the experiment. Therefore, its contribution to the calculation of maintenance coefficient was negligible when compared with the other term, $F(C_i - C_f)$, being approximately 324 mg/h (see Eq. 7).

Dynamics of the maintenance coefficient

The assumption of Pirt's method is that maintenance coefficient and true cell growth yield are constant. According to the definition of maintenance coefficient in this study, however, it should be closely associated with culture conditions and cell growth rate or specific growth rate. Therefore, many researchers including Pirt himself later indicated that maintenance coefficient may not be always constant and related it with specific growth rate [18, 24, 28–30]. In the present study, maintenance coefficient may not be constant during transient state in which cell concentration and specific growth rate continuously change.

In order to calculate the maintenance coefficient, it is first necessary to obtain an estimate of the true cell growth yield, as shown in Eq. 7. However, as will be discussed later, the true cell growth yield may not be constant but may vary in a narrow range. We selected some values to investigate the dynamics of maintenance coefficient and ultimate maintenance coefficient at biological steady state. Instead of choosing arbitrary values, values were adopted from the observed cell growth yield in a single aqueous phase [27], 0.46, the observed cell growth yield in a TPPB [7], 0.56, true cell growth yield in the range of interest, 0.69 and true cell growth yield throughout the experiment [20], 0.82, respectively. Since the true cell growth yield is greater than the observed one, the actual true cell growth yield may lie between 0.56 and 0.82. Maintenance coefficients were calculated using Eq. 7 and the dynamics are shown in Fig. 3, which indicate that the initial maintenance coefficients at time zero are largest, and larger with higher cell growth yield. The reason for high initial values of maintenance coefficient may be attributed to the high cell activity and proliferation which requires more energy for motility, active transport of molecules and synthesis of macromolecules such as RNA and proteins. The maintenance coefficients converged to a specific value within 200 h, 1.750×10^{-2} h^{-1}, regardless of the cell growth yields. In fact, this value itself is also easily predicted from Eq. 10 in which only cell concentration at biological steady state is required as a biological factor in the maintenance coefficient calculation. Thus, it should be noted that some errors in estimating true cell growth yield would not lead to a noticeable deviation in maintenance coefficient estimate at biological steady state. According to Eq. 7, the maintenance coefficient can be determined using cell concentration and specific growth rate data. In a chemostat, maintenance coefficient decreases with an increase of specific growth rate or dilution rate at steady state [24, 25]. However, unlike in chemostat cultures, cells in the TPPB are retained and accumulate, a condition which complicates the ability to predict the general effect of cell concentration or specific growth rate on maintenance coefficient since, the maintenance coefficient increases with decreasing specific growth rate or cell concentration. As already shown in Fig. 2 and Eq. 13, specific growth rate

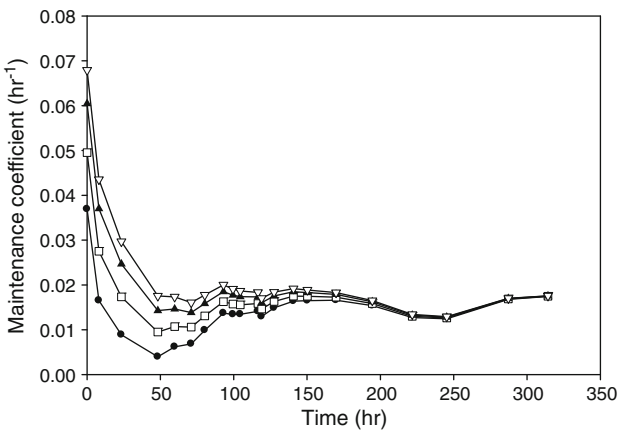

Fig. 3 Dynamics of maintenance coefficient ($Y_{x/s}$: *filled circle* 0.46, *open square* 0.56, *filled triangle* 0.69, *open inverted triangle* 0.82)

is closely related to cell concentration, and shows an opposite trends with time, that is, the increase in cell concentration in the TPPB causes specific growth rate to decrease. The fluctuation of maintenance coefficient observed in the Fig. 3 is mathematically the result of the effects of cell concentration and specific growth rate. The maintenance coefficients finally converge to a specific value when cell concentration is constant and specific growth rate is zero, which is the biological steady state.

As for true cell growth yield, although most of the theoretical approaches for kinetic studies assume that the true cell growth yield is constant for a given substrate [25], it would be more reasonable to say that true cell growth yield could not always be constant, which would make the estimation of maintenance coefficient, impossible because we would need to know true cell growth yield to find maintenance coefficient or vice versa. In a paper with a different microorganism and with acetic acid as a carbon and energy source being used, it was suggested that both maintenance energy and true cell growth yield change with temperature in the range 20–32.5 °C but true cell growth yield varied within only 20% while maintenance coefficient varied 1,100% [19]. Pipyn and Verstraete [31] also indicated that true cell growth yields ranged from 0.5 to 0.8 for different microorganisms, substrates and temperatures while maintenance coefficients varied in the range 0.01–0.14 h^{-1}. Since temperature and pH were strictly controlled at 30 °C and 6.6, respectively [20], the true cell growth yield may have varied in a narrow range. In addition, since all the benzene consumption related to many physiological phenomena other than cell growth are lumped into maintenance, true cell growth yield can be said to be associated with only cell mass whose composition does not vary much as long as other components are present sufficiently and no substantial by-products are formed. On this basis, we tested two values of 0.69 and 0.82 as will be discussed later and found that 0.69 was more appropriate to describe the actual cell concentration change. However, it should again be noted that true cell growth yield may change slightly in a TPPB but its change may be minor compared to that of maintenance coefficient. We would acknowledge that 0.69 is not necessarily the true value but a practical value of true cell growth yield for the calculation of maintenance coefficient.

The cell concentration during TPPB operation was calculated with the values of maintenance coefficient and true cell growth yield (0.69 and 0.82) suggested in this study. We also performed the same calculation with the constant maintenance coefficient of 1.670×10^{-2} h^{-1} and true yield of 0.69 suggested by Nielsen et al. [20]. The comparison is shown in Fig. 4 and indicates that the three methods exhibit a very similar trend and that the case of the variable maintenance with constant true growth yield of

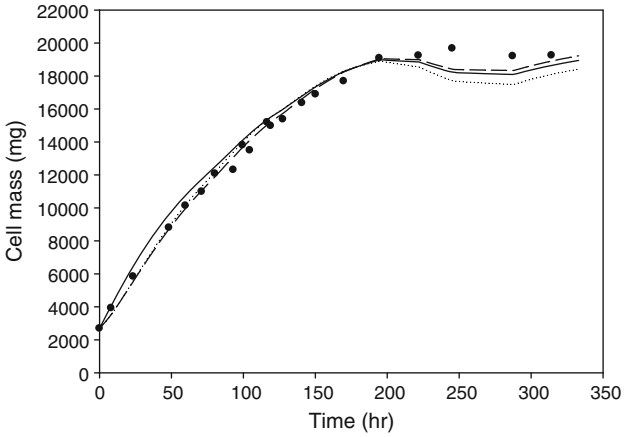

Fig. 4 Comparison of estimated cell mass with actual one in the TPPB. *Solid circle* represents actual cell mass. *Solid line* represents constant maintenance coefficient (1.670×10^{-2} h^{-1}) and true cell growth yield (0.69), *dashed line* for varied maintenance coefficient and constant true cell growth yield of 0.69, and *dotted line* for varied maintenance coefficient and constant true cell growth yield of 0.82

0.69 is in a bit better agreement with actual cell mass. It should be noted that the maintenance coefficient is variant but can be practically treated in some period as a constant in this study because its change is small after 100 h of operation. Therefore, the assumption of constant maintenance coefficient (1.670×10^{-2} h^{-1}) and true cell growth yield (0.69) suggested by Nielsen et al. [20] can be said to be substantially valid in the TPPB and this indirectly justified the true cell growth yield of 0.69. Additionally, it should be noted that the far right term of Eq. 7 comprised of specific growth rate and true cell growth yield contribute only a small amount to the calculation of maintenance coefficient because specific growth rate is too small, in particular, after 100 h of operation, which made Eq. 7 close to Eq. 10. Consequently, the total maintenance energy, maintenance coefficient multiplied by cell mass, for the three cases shows very similar behaviour except for early operation as shown in Fig. 5. The decrease of estimated cell mass during 220–310 h in Fig. 4 was due to noticeable decrease of inlet benzene concentration to around 4.5 mg/L and this temporary shortage of benzene supply caused insufficiency even for the demand of maintenance by the cells present as shown in Fig. 5.

The existence of a biological steady state has been observed in other TPPB operations. According to Aldric and Thonart [11], *Roodococcus erhthropolis* T902.1 reached biological steady state in a TPPB composed of water and silicone oil to treat isopropylbenzene. The cell mass was maintained in the range of 9–11 g/L for 10 days and the fluctuation of cell mass may be due to intermittent loading of the compound for 10 h a day and 5 days a week to simulate an actual industry operating schedule. Interestingly, other research using polymers as a second phase

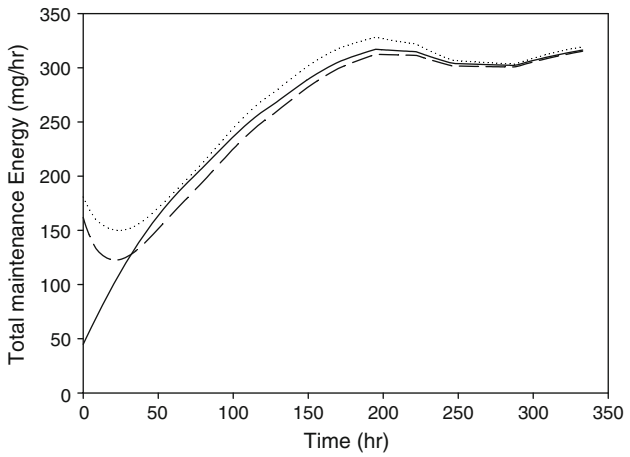

Fig. 5 Comparison between estimated total maintenance energies from three different assumptions in the TPPB. *Solid line* represents constant maintenance coefficient (1.670×10^{-2} h^{-1}) and true cell growth yield (0.69), *dashed line* for varied maintenance coefficient and constant true cell growth yield of 0.69, and *dotted line* for varied maintenance coefficient and constant true cell growth yield of 0.82

instead of an organic solvent in a TPPB fed with a mixture of benzene, toluene, ethylbenzene and *o*-xylene to a microbial consortium showed biological steady state from 300 to 400 h of operation with a collective maintenance coefficient of 0.0103 h^{-1} at biological steady state [9]. We could prolong the biological steady state to about 1 month without medium change by refining medium formulation with the same microorganism, *A. xylosoxidans* Y234, for benzene degradation in a TPPB [32]. According to the above study, no intermediate accumulation was found during the biological steady state and the concentration of benzene in the aqueous phase was far below the inhibition level as already shown previously [20, 26, 32]. It was also observed that the number of viable cells was almost constant during the state, and the uptake rate of the nitrogen source at the biological steady state was 5–6 times lower than that during active growth for the same benzene elimination rate. These phenomena may indicate that metabolic activities predominantly exist in a 'maintenance state' during which most of the cellular activity is directed towards energy generation with minimal biosynthesis [32]. On the basis of these observations, it is thought that the cell growth rate or cell division is extremely low at the biological steady state, indicated by the low maintenance coefficient. Consequently, a balance between minor cell growth and death could result in the constant cell mass observed, corresponding to biological steady state.

The maintenance coefficient at biological steady state is an intrinsic value for a microorganism on a given substrate [20]. Using Eq. 10, we can easily calculate the relationship between cell concentration and inlet benzene concentration or inlet loading rate. If we set removal efficiency to be 99% and fix the volume of the aqueous phase to be 2 L and the gas flow rate at 60 L/h, Eq. 10 can be rearranged as follows:

$$X_A = 1697.4 \times C_i \quad (14)$$

This equation implies that a TPPB can theoretically treat benzene at as high a concentration as is desired as long as cell mass in the aqueous phase can be met at biological steady state. For example, 5.5 mg/L of inlet benzene can be successfully treated with 99% of removal efficiency as long as 9,268 mg/L of cell concentration is maintained at biological steady state (see also Fig. 2). However, there might be other limiting factors, violating the assumption of this study, which decrease the performance of the TPPBs or even lead to process failure. The likely factor may be dissolved oxygen in the TTPB. Therefore, it is necessary to compare the oxygen requirement with oxygen supply in the TPPB [8]. The oxygen demand is proportionally dependent on total cell mass which is determined by maintenance coefficient while oxygen supply can be controlled by operating conditions. Therefore, the possibility of oxygen limitation under various operation conditions should be examined carefully as discussed in a previous study [8].

Roles of microorganism in the design of TPPBs

It can be generally assumed that the microorganisms used in a TPPB, either pure or mixed culture, have the ability to degrade the target VOCs ensuring that the system is not biodegradation limited. That is, the microorganisms should degrade the pollutants at acceptably high rates and have lower half saturation and higher inhibition constants in their kinetics model such as that described by Andrews [8, 33].

We have prepared a simple diagram to show the role of microorganisms in TPPB design and operation as shown in Fig. 6. In developing TPPBs, it is usually appropriate to first characterise the properties of the cells that will be used to degrade the target pollutants. To do this, it is possible to measure cell mass and pollutant concentrations in the aqueous and organic phases in the TPPBs during operation, which allows calculation of the specific growth rate and observed cell growth yield. The half saturation and inhibition constants are usually estimated separately in batch experiments and these constants play relatively minor roles at biological steady state as long as the TPPBs are mass transfer limited, although they can be important in transient loadings during TPPB operation. As stated above, the maintenance coefficient at transient state can be estimated using Eq. 1 and this is easily calculated at biological steady state using Eq. 10. Although inlet and outlet concentrations of the pollutants are required for the calculation of the maintenance coefficient at biological steady state, the maintenance coefficient is constant at biological steady

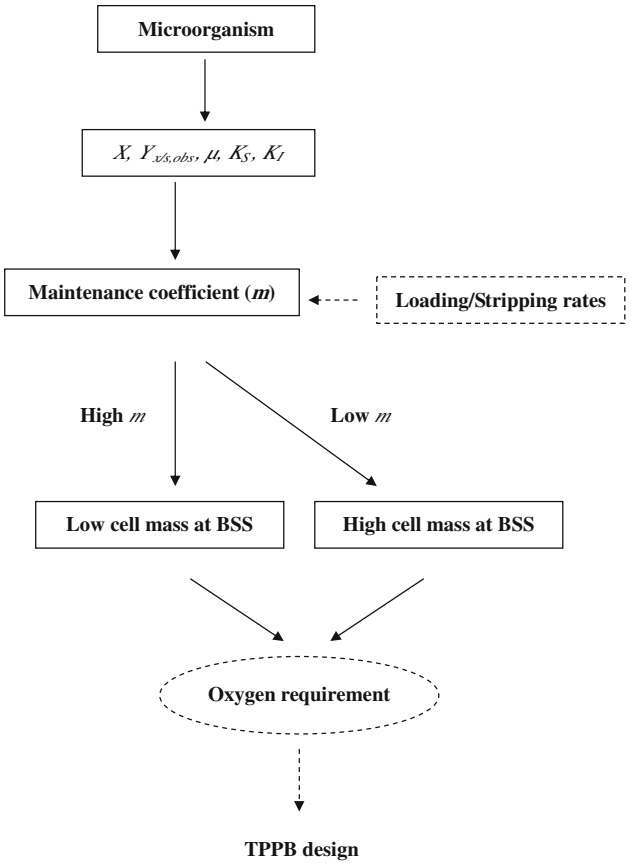

Fig. 6 Microorganisms as the director to the design of TPPBs

state, regardless of pollutant concentrations. According to the definition of maintenance coefficient, high maintenance coefficients imply that low cell mass is required and maintained at the biological steady state. Cells with high maintenance coefficients would be favourable in the TPPBs operation because reduced cell mass could lead to the requirement for less mineral medium, a smaller working volume, and consequently lower operating cost.

The notion of maintenance coefficient could also be applied to biofilter design. As is generally recognised, cell mass control is a challenging problem in biofilter operation, and the immobilized cell mass above some level is not effective any more in terms of the degradation rate of pollutants because of mass transfer limitations and reduction of available surface area in a biofilter [34]. Accordingly, cells with high maintenance coefficient would be preferable in biofilter operation because this prolongs the interval between cell removals or reduces the necessity of cell removal completely.

Cells have their own specific rate of oxygen consumption for maintenance [35] and the cell mass at biological steady state directly determines the requirement of oxygen, which could be a limiting factor in TPPBs [8]. Therefore, the oxygen requirement is an important factor that determines operating conditions of the TPPBs and optimum operating conditions including portion of organic phase, aeration rate and agitation speed as described in a previous study [8]. Accordingly, key microbial properties, maintenance coefficient and specific rate of oxygen consumption for maintenance, determine cell mass and oxygen requirement at biological steady state, which makes the microorganisms play a central role in the design and operation of TPPBs.

Conclusions

In the development of TPPBs, the portion of organic phases has been found to be a crucial factor because it determines the enhancement of oxygen transfer into the TPPB. Since oxygen supply should meet the demand in TPPBs, which is proportionally dependent on the cell mass, the estimation of cell mass requirement to treat a given inlet loading rate of VOCs should be determined. The maintenance coefficient, calculated using a material balance equation, allows calculation of the cell mass requirement at biological steady state. Through the calculation of the dynamics of maintenance coefficient, it was found that it was initially highest when cells are actively growing and gradually decreased. The maintenance coefficient, though fluctuating, converged to a specific value, 1.750×10^{-2} h^{-1} in the case of *A. xylosoxidans* on benzene at biological steady state at which point no net cell increase was observed. The cell mass requirement at biological steady state for a given loading rate can be obtained from the maintenance coefficient, and the oxygen demand of the TPPB can be easily calculated using the cell mass and specific rate of oxygen consumption for maintenance.

Since high maintenance coefficient implies that less cell mass is required at biological steady state, high maintenance would be favourable in TPPBs operation because it would lead to less mineral medium, less working volume requirements and consequently lower operating cost. Since cell mass control is a challenging problem in biofilters as well as TPPBs, we would like to suggest that researchers should investigate the maintenance coefficient of candidate cells in advance when choosing the optimum microorganisms.

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