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EFFECT OF INITIAL SUBSTRATE/INOCULUM RATIO ON CELL YIELD IN THE REMOVAL OF HYDROPHOBIC VOCS IN FUNGAL BIOFILTERS

EFECTO DE LA RAZÓN SUSTRATO/INÓCULO SOBRE EL RENDIMIENTO CELULAR EN LA REMOCIÓN DE COVS HIDROFÓBICOS EN BIOFILTROS FÚNGICOS

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Abstract

Different kinetic models have been proposed to describe the elimination of hydrophobic volatile organic compounds (VOCs) by fungal biofiltration. In this process the ratio of the initial substrate concentration (C^p_{b0}) to the initial biomass (X_0) has been shown to influence the cell yield. This paper presents a study of the effect of the C^p_{b0}/X_0 ratio on observed cell yield (Y_{obs}) in a fixed bed batch system (microcosm) using a gaseous carbon source, as an approximation to its application in the fungal biofiltration of hydrophobic VOCs. Essays were carried out in fixed-bed microcosms using the filamentous fungus *Fusarium solani* as a biological agent and *n*-pentane as a carbon and energy source. The results indicated that Y_{obs} in the gas phase is inversely proportional to the C^p_{b0}/X_0 ratio, with values of 0.9 to 0.35 $g_{biomass}\ g^{-1}_{pentane}$ being obtained when the C^p_{b0}/X_0 ratio is changed from 0.1 to 1.0 $g_{pentane}\ g^{-1}_{biomass}$. The results indicate that more than 60% of *n*-pentane was consumed due to energy spilling, and that strong dissociation of catabolism from anabolism occurred at higher C^p_{b0}/X_0 ratios.

Keywords: hydrophobic VOCs, fungal biofiltration, substrate/inoculum ratio, growth yield.

Resumen

Diferentes modelos cinéticos han sido propuestos para describir la eliminación de compuestos orgánicos volátiles hidrofóbicos (COVs) en biofiltros fúngicos. En este proceso la razón de la concentración inicial de sustrato (C^p_{b0}) a la biomasa inicial (X_0) ha mostrado influir en el rendimiento celular. Este artículo presenta el estudio del efecto de la razón C^p_{b0}/X_0 en el rendimiento celular observado (Y_{obs}) en un sistema por lote de lecho fijo (microcosmo) utilizando una fuente de carbono gaseosa, como una aproximación a su aplicación en la biofiltración fúngica de COVs hidrofóbicos. Los ensayos fueron realizados en microcosmos de lecho fijo utilizando el hongo filamentoso *Fusarium solani* como agente biológico y *n*-pentano como fuente de carbono y energía. Los resultados indican que Y_{obs} en la fase gaseosa es inversamente proporcional a la razón C^p_{b0}/X_0, con valores de 0.9 a 0.35 $g_{biomasa}\ g^{-1}_{pentano}$ siendo obtenido cuando la razón C^p_{b0}/X_0 es cambiada desde 0.1 a 1.0 $g_{pentano}\ g^{-1}_{biomasa}$. Los resultados indican que más del 60% del *n*-pentano fue consumido debido a pérdida de energía, y que una fuerte disociación del catabolismo y anabolismo ocurre para altas razones C^p_{b0}/X_0.

Palabras clave: COVs hidrofóbicos, biofiltración fúngica, razón sustrato/inóculo, rendimiento de crecimiento.

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1 Introduction

Fungal biofiltration is a biological technology used for the elimination of hydrophobic VOCs. It offers several comparative advantages over other systems, such as the high hydrophobicity of the fungus surface, a large transport area due to the presence of aerial hyphae, and feasibility for use with high gas flows at low VOCs concentrations (Vergara-Fernández *et al*. 2006 and 2011a; Vigueras *et al*. 2009; Rene *et al*. 2010).

The effect of the VOCs concentration and start-up on the elimination capacity of biofiltration systems has been extensively studied in different mathematical models representing the operation of the fungal biofilter (Maestre *et al*. 2007; Devinny and Ramesh, 2005; Spigno *et al*. 2003; Vergara-Fernández *et al*. 2008; González-Vázquez *et al*. 2011). However, the relationship between them, and the effect of this ratio on cell yield, have not been sufficiently studied for the removal of hydrophobic VOCs in fungal biofilters. The different mathematical models used in biofiltration systems inoculated with bacteria and filamentous fungi have used cell yield as a constant parameter (Vergara-Fernández *et al*. 2008; Arriaga and Revah, 2009; Spigno and De Faveri, 2005; Dorado *et al*. 2008). This is because the models developed for the biofiltration of gases only consider the cell yield for the substrate (VOCs) consumed in biomass growth, and do not include substrate consumption due to cell maintenance, product formation, heat loss, etc. (Liu and Guang-Hao, 1997). Moreover, the presence of fungal biomass in biofiltration systems causes an increase in the solubility of hydrophobic VOCs (Vergara-Fernández *et al*. 2006), generating excess substrate with respect to the other nutrients presents, which must cause strong uncoupling between anabolism and catabolism leading to energy spilling. This has not previously been considered, since traditionally the cell yield obtained in the liquid medium has been used, being one of the main reasons it is difficulty measuring the presence of the other nutrients in the medium.

For many years it was assumed that living organisms always utilized ATP in a highly efficient manner, but simple growth studies with bacteria indicated that the efficiency of biomass production was often at least 3-fold lower than the amount that would be predicted from standard biosynthetic pathways. These ideas and thermodynamic arguments indicated that cells might have another avenue of energy utilization. This phenomenon has also been called 'uncoupling', 'spillage' and 'overflow metabolism', but 'energy spilling' is probably the most descriptive term (Russell, 2007).

Although cell yield has been considered as a constant value, some studies in the liquid phase using batch and continuous cultures have suggested that cell yield varies according to the ratio between the initial substrate and the initial biomass (Liu, 1996; Liu *et al*. 1998; Liu *et al*. 2005; Wang *et al*. 2007). Vergara-Fernández *et al*. (2008) developed a phenomenological model with experimental validation for *n*-hexane fungal biofiltration; they noted a deviation between the results in the simulation and the experiment which shows a change in the cell yield from 0.1 to 0.8 for model validation. This indicates the need for a model to establish cell yield variations for biofiltration systems under different conditions of substrate and biomass concentrations. On the other hand, it is important to consider that in biofiltration systems is undesirable to have high yields of biomass and accumulation reservation molecules, as this could clog the system; however it is desirable to have high values of energy dissipation.

The influence of the substrate/inoculum ratio also has been studied to determine its effect on methane yield in the anaerobic digestion of different raw materials such as bean curd refuse-okara (Zhou *et al*. 2011) and *Microcystis* spp. (Zeng *et al*. 2010). Furthermore, Aktas (2012) determined the effect of the substrate/inoculum ratio on the biodegradation of phenolic compounds in an activated sludge, indicating that the kinetic parameters obtained in a batch reactor can reflect the biomass activity in continuous-flow reactors. Wang *et al*. (2007) studied the effect of the ratio on the biomass growth of *Ralstonia eutropha* for the production of polyhydrobutyrate (PHB).

The aim of this study is to show the effect of the ratio between the initial concentrations of hydrophobic VOCs/fungal biomass (C^p_{b0}/X_0) on the observed growth yield (Y_{obs}) in a fixed bed batch system. For this, tests were conducted on microcosms in solid media inoculated with *Fusarium solani* and fed with *n*-pentane as the carbon source. Cell yields observed at different values of substrate-inoculum ratio were adjusted to energy uncoupling model that accounts for this phenomenon.

2 Materials and methods

2.1 Microorganisms and microcosm preparation

Fusarium solani B1 was grown in solid media (perlite imbibed with liquid mineral medium) in closed environments (microcosms). The experiments were performed in triplicate in 125 mL serum bottles sealed with Mininert Teflon Valves (VICI; Precision Sampling, Baton Rouge, LA).

Series of batch experiments were carried out at different C^p_{b0}/X_0 ratios with initial *n*-pentane concentrations (C^p_{b0}) in wet biomass between 22 and 435 g m^{-3}. The range of values obtained for C^p_{b0} correspond to initial *n*-pentane headspace concentrations (C^p_{h0}) between 0.5 and 10 g m^{-3} (range of concentrations commonly used in biofiltration systems for removing hydrophobic VOCs) (Dorado *et al*. 2008; Arriaga and Revah, 2009; Vergara-Fernández *et al*. 2012). The C^p_{h0} was determined by direct gas chromatography injection. In addition C^p_{b0} was obtained by *n*-pentane/biomass partition coefficient (K^w_b) as:

$$K^w_b = \frac{C^P_{h0}}{C^P_{b0}} \tag{1}$$

The partition coefficient for the biomass on a wet basis at 25 ºC (K^w_b = 0.023) was obtained from Vergara-Fernández *et al*. (2011b). Control experiments showed negligible sorption of *n*-pentane on perlite.

To evaluate the observed cell yield (Y_{obs}), 1.5 g support (perlite) was mixed with 10 mL of mineral medium containing an initial active fungal mycelium (initial biomass concentration, X_0) at 0.35 $g_{biomass}$ L^{-1} (equivalent to 2.33 $mg_{biomass}$ $g^{-1}_{\text{dry perlite}}$). This was placed in 125 mL serum bottles for all batch tests and a desired C^p_{b0} /X_0 ratio was obtained by varying the initial substrate concentration. Batch tests were carried out at 25 ºC.

2.2 Carbon source and mineral medium

The model compound used as the carbon source was *n*-pentane (Merck, 99%). Mineral medium was prepared in a buffered phosphate solution (pH 4) and contained (g L^{-1}): 18 $NaNO_3$; 1.3 KH_2PO_4; 0.38 $MgSO_4{\cdot}7H_2O$; 0.25 $CaSO_4{\cdot}2H_2O$; 0.055 $CaCl_2$; 0.015 $FeSO_4{\cdot}7H_2O$; 0.012 $MnSO_4{\cdot}H_2O$; 0.013 $ZnSO_4{\cdot}7H_2O$; 0.0023 $CuSO_4{\cdot}7H_2O$; 0.0015 $CoCl_2{\cdot}6H_2O$; 0.0015 H_3BO_3.

2.3 Energy uncoupling model

The C^p_{b0} /X_0 ratio represents the availability of carbon and energy source for microbial growth in a batch culture. To determine the effect of the C^p_{b0} /X_0 ratio on the yield in a batch culture, the Y_{obs} were adjusted according to the model developed by Liu (2000) (Eq. 2), considering the substrate consumed due to growth, cell maintenance and energy spilling.

$$\frac{1}{Y_{obs}} = \frac{1}{(Y_{obs})_{\max}} + \frac{1}{(Y_w)_{\min}} \frac{C^P_{b0}/X_0}{C^P_{b0}/X_0 + K_{S/X}} \tag{2}$$

where Y_{obs} is the observed cell yield, $(Y_{obs})_{\max}$ is the observed growth yield of substrate-limited culture, $(Y_w)_{\min}$ is the minimum energy spilling-related cell yield and $K_{S/X}$ is the C^p_{b0} /X_0 ratio-related saturation constant. Eq. (2) shows that the ratio between anabolism and catabolism is taken to be dependent on the C^p_{b0} /X_0 ratio (Liu *et al*. 1998). Following Liu *et al*. (1998), the concept of the energy uncoupling coefficient is used in this work to describe the uncoupling observed between anabolism and catabolism under substrate-sufficient conditions (Eq. 3).

$$E_u = \frac{(Y_{obs})_{\max} - Y_{obs}}{(Y_{obs})_{\max}} \tag{3}$$

where E_u is the energy uncoupling coefficient. This parameter features reduction in the efficiency of converting energy into cell biosynthesis under substrate-sufficient conditions. Substituting Eq. (2) into Eq. (3) produces the C^p_{b0} /X_0 -dependent expression for the energy uncoupling coefficient:

$$E_u = E_{u,\max} \frac{C^P_{b0}/X_0}{C^P_{b0}/X_0 + K^*_{S/X}} \tag{4}$$

where

$$E_{u,\max} = \frac{(Y_{obs})_{\max}}{(Y_{obs})_{\max} + (Y_w)_{\min}} \tag{5}$$

$$K^*_{S/X} = \frac{(Y_w)_{\min}}{(Y_{obs})_{\max} + (Y_w)_{\min}} K_{S/X} \tag{6}$$

where $E_{u,max}$ is the maximum energy uncoupling coefficient and $K^*_{S/X}$ is the yield-related saturation constant.

2.4 Analytical methods

The gaseous *n*-pentane concentration was measured in triplicate by FID-GC, Shimadzu 2014 (detection temperature 220ºC, injection temperature 80ºC and column temperature 200ºC), equipped with a capillary

column, model Rtx-5 Restex UE (30 m × 0.32 mm × 0.25 μm), using nitrogen as a carrier gas.

The biomass was measured as volatile solids with a thermogravimetric analyzer according to Arriaga and Revah (2005). This analysis allowed the mass losses to be quantified and associated with the processes of water and carbon combustion. Measurements were done in duplicate.

3 Results and discussion

Fig. 1 shows the experimental values of Y_{obs} obtained for different values of the C^P_{b0}/X_0 ratio using *F. solani* grown in *n*-pentane gas. The experimental values of Y_{obs} were adjusted to the mathematical model proposed by Liu (2000) based on the C^P_{b0}/X_0 ratio (Eq. 2). Thus, the parameters of the model were $(Y_{obs})_{\max}$ = 0.96 $g_{biomass}\ g^{-1}_{pentane}$; $(Y_w)_{\min}$ = 0.12 $g_{biomass}\ g^{-1}_{pentane}$ and $K_{S/X}$ = 2.71 $g_{pentane}\ g^{-1}_{biomass}$.

As shown in Fig. 1, Y_{obs} decreased by a factor of three when the C^P_{b0}/X_0 ratio was increased by a factor of ten, becoming approximately constant for a C^P_{b0}/X_0 ratio higher than 0.5 ($Y_{obs} \approx 0.35$), while for ratios between 0.1 and 0.5 a sharp decline in Y_{obs} is observed, from 0.9 to 0.35, respectively. Reduced cell yields show dissociation of anabolism from catabolism. In fact, much research shows that under substrate-sufficient conditions, Y_{obs} decreases significantly with increasing residual substrate concentration for continuous and batch culture (Yamame *et al.* 1992; Liu, 1996; Liu and Guang-Hao, 1997).

The range of Y_{obs} obtained (between 0.9 to 0.35 $g_{biomass}\ g^{-1}_{pentane}$) in this work was similar to values reported by Vergara-Fernández *et al.* (2008) (0.1 - 0.8 $g_{biomass}\ g^{-1}_{hexane}$) in the simulation of *n*-hexane fungal biofiltration using the fungus *Fusarium solani*. In comparable works, Macris and Kokke (1978) reported values of 0.71 $g_{biomass}\ g^{-1}_{\text{carob sugar}}$ using the fungus *Fusarium moniliforme*, while Shellart (1975) and Lareo *et al.* (2006) reported average values of 0.54 $g_{biomass}\ g^{-1}_{substrate}$ using the fungi *Trichoderma viride* with corn waste effluent substrate, and *Mucor bacilliformis* with glucose, respectively.

On the other hand, Garnier *et al.* (1999), using *n*-pentane as the carbon source, reported values of 0.9 $g_{biomass}\ g^{-1}_{pentane}$ with *Pseudomonas aeruginosa*; while Takahashni (1970), using bacteria from a soil sample, reported values of 0.85 $g_{biomass}\ g^{-1}_{pentane}$. Miller and Johnson (1966), using yeast, reported values of 0.8 $g_{biomass}\ g^{-1}_{pentane}$.

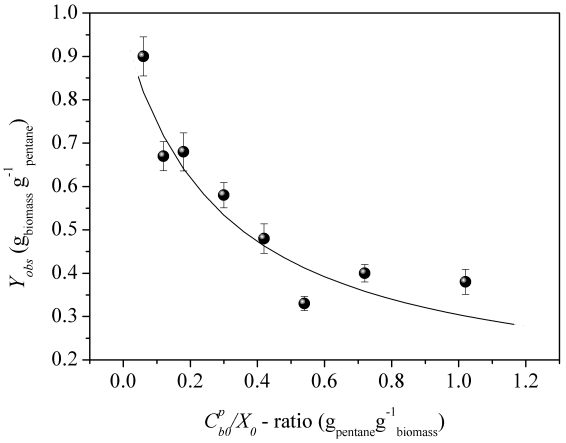

Fig. 1 Relationship between Y_{obs} and $/X_0$ ratio for *Fusarium solani* grown on *n*-pentane gas. (•) Experimental data; (—) model prediction (Eq. 2). $(Y_{obs})_{max}$ = 0.96 $g_{biomass}\ g^{-1}_{pentane}$; $(Y_w)_{min}$ = 0.12 $g_{biomass}\ g^{-1}_{pentane}$ and $K_{S/X}$ = 2.71 $g_{pentane}\ g^{-1}_{biomass}$.

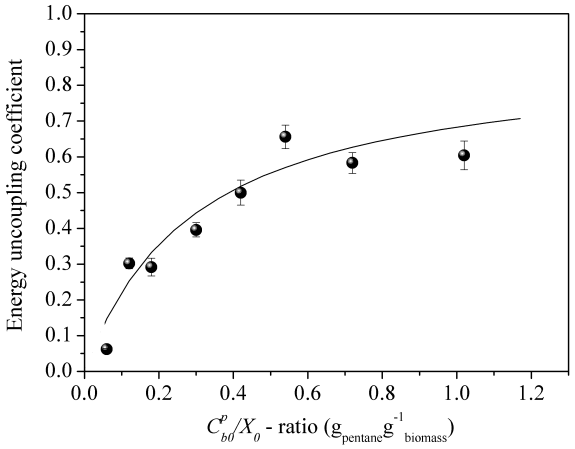

Fig. 2 Relationship between the C^p_{b0}/X_0 ratio and the energy uncoupling coefficient for *Fusatium solani* grown on *n*-pentane gas. (•) Experimental data; (—) model prediction (Eq. 4). $E_{u,max}$ = 0.89; $K^*_{S/X}$= 0.30 $g_{pentane}\ g^{-1}_{biomass}$.

Fig. 1 shows that the mathematical model fitted the experimental data, proving that the effect of the C^P_{b0}/X_0 ratio reported by Liu (2000) in liquid medium bioreactors can be extrapolated to fixed bed bioreactors with a gaseous carbon source.

Fig. 2 shows a comparison between the observed E_u and the values obtained using Eq. (4) indicating that the model proposed by Liu *et al.* (1998) for substrate-sufficient batch culture of activated sludge microorganisms can be adequately applied to

a gaseous carbon source in a fixed bed bioreactor batch culture inoculated with filamentous fungus. The parameters of the model (Eq. 4) were $E_{u,max} = 0.89$ and $K^*_{S/X} = 0.30$ $g_{pentane}$ $g^{-1}_{biomass}$.

Fig. 2 shows that the energy uncoupling coefficient reaches 0.6 when the C^P_{b0}/X_0 ratio is greater than 0.5 $g_{pentane}$ $g^{-1}_{biomass}$. A similar energy uncoupling coefficient of 0.65 was observed by Chang *et al.* (1993) at C^P_{b0}/X_0 ratios greater than 10 mg_{COD} mg^{-1}_{MLSS}, while Liu *et al.* (1998) reported an energy uncoupling coefficient value of 0.5 using a batch culture of activated sludge.

The results obtained in this study indicate that more than 60% of *n*-pentane was consumed due to energy spilling, and that a strong dissociation of catabolism from anabolism occurred at higher C^P_{b0}/X_0 ratios. These results show that at higher C^P_{b0}/X_0 ratios, bioenergy may be produced faster than it would be used by microorganisms, and this result is favoured by increasing the solubility of *n*-pentane on the hydrophobic surface of the fungus.

The results of Figs. 1 and 2 fit the changing Y_{obs} obtained by Vergara-Fernández *et al.* (2008) for a *n*-hexane biofiltration system. In this system, low Y_{obs} values (0.1 $g_{biomass}$ g^{-1}_{hexane}) are found in the latency stage due to low biomass concentrations (high C^P_{b0}/X_0 ratio), while after the steady state was reached the higher Y_{obs} (0.8 $g_{biomass}$ g^{-1}_{hexane}) represents a high intake of biomass for the same amount of carbon source (low C^P_{b0}/X_0 ratio). This effect is consistent with the findings of Wang *et al.* (2007), Liu *et al.* (2005) and Liu (2000), and demonstrates that the physiological uncoupling of catabolism and anabolism extends to biofiltration processes with a gaseous carbon source.

Carbon balance results of about 50% to 70%, considering cell maintenance, biomass growth and mineralization of the carbon source, have been obtained in different works on fungal biofiltration of VOCs, in which the rest of the carbon and energy source has not been clearly quantified (Arriaga and Revah, 2009; Vergara-Fernández *et al.* 2012; Vergara-Fernández *et al.* 2011b; Hernández-Meléndez *et al.* 2008). These results in continuous culture biofiltration systems indicate that the energy loss due to uncoupling may be up to 50%, similar to the 60% maximum obtained in this work in a fixed bed batch system using a gaseous carbon source. These results indicate that it is feasible to use microcosm results to determine the effect of the C^P_{b0}/X_0 ratio on fungal biofiltration of hydrophobic VOCs.

These results indicate the feasibility of determining C^P_{b0}/X_0 ratios that allow decreasing the amount of biomass generated and possible accumulation of material in long periods of a biofilter operation, so as to prevent possible clogging and maximizing the energy dissipation source of carbon.

Conclusions

The results indicate that it is reasonable to consider the initial substrate concentration normalized to the initial biomass concentration (C^P_{b0}/X_0 ratio) to describe the availability of carbon and energy for fungal growth in the modeling of a biofilter hydrophobic VOCs. These results indicate that the C^P_{b0}/X_0 ratio has an effect on Y_{obs} in fixed bed systems using a gaseous carbon source. In this case, when *F. solani* is fed with a gaseous carbon source (*n*-pentane) in a fixed bed system, the effect observed is similar to that reported in the liquid phase, show maximum energy uncoupling of 60%.

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Nomenclature

C^P_{b0}	initial *n*-pentane concentration in wet biomass $g_{pentane}$ $m^{-3}_{\text{wet biomass}}$
C^P_{h0}	initial *n*-pentane headspace concentration $g_{pentane}$ $m^{-3}_{headspace}$
E_u	energy uncoupling coefficient
$E_{u,max}$	maximum energy uncoupling coefficient
K^w_b	*n*-pentane/biomass partition coefficient $g_{pentane}$ $m^{-3}_{\text{wet biomass}}$ / $g_{pentane}$ $m^{-3}_{headspace}$
$K_{S/X}$	C^P_{b0}/X_0 ratio-related saturation constant $g_{pentane}$ $g^{-1}_{biomass}$
$K^*_{S/X}$	yield-related saturation constant $g_{pentane}$ $g^{-1}_{biomass}$
Y_{obs}	observed growth yield $g_{biomass}$ $g^{-1}_{pentane}$

$(Y_{obs})_{max}$	observed growth yield of substrate-limited culture $g_{biomass}\ g^{-1}_{pentane}$
$(Y_w)_{min}$	minimal energy spilling-related growth yield $g_{biomass}\ g^{-1}_{pentane}$
X_0	initial biomass concentration $g_{biomass}\ m^{-3}_{\text{dry perlite}}$

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