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Required equilibrium studies for designing a three-phase bioreactor to degrade trichloroethylene (TCE) and tetrachloroethylene (PCE) by *Trametes versicolor*

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Abstract

This paper aims to investigate the three-phase equilibriums to design a bioreactor to degrade trichloroethylene (TCE) and tetrachloroethylene (PCE) by the white-rot fungus *Trametes versicolor*. The vapour–liquid equilibrium constants were obtained from bibliographic values and these values are 0.392 for TCE and 0.723 for PCE, which show that these two compounds are very volatile. Adsorption of TCE and PCE by dead biomass pellets was studied. Due to the low concentration range studied for both compounds, the linear equation is enough to describe the adsorption equilibrium. The adsorption parameters are 0.110 and 0.176 for TCE and PCE, respectively. Then the equilibrium information obtained was used to calculate the distribution of these contaminants in a three-phase bioreactor in specific degradation conditions and despite it is checked that adsorption is not very high, it is significant (between 12.0% and 12.8%). Moreover, a comparison between TCE and PCE experimental and calculated degradation yields was done to validate the linear isotherm.

Keywords: Trichloroethylene; Tetrachloroethylene; *Trametes versicolor*; Adsorption; Degradation

1. Introduction

Trichloroethylene (TCE) and tetrachloroethylene (PCE) are halogenated organic compounds, which have been widely used in industrial cleaning solutions. TCE and PCE are ranked sixteenth and thirty-first, respectively, on the 2005 Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, commonly known as Superfund) priority list for hazardous substances because they are toxic widespread pollutants (http://www.atsdr.cdc.gov/cercla/).

Physical treatments, which consist of a phase change of the contaminant and a subsequent treatment to eliminate it, have been extensively used for TCE and PCE removal. Two of the most used physical treatments are carbon adsorption and stripping methods [1,2].

Chemical treatments, which are based on oxidation processes, have been suggested as a suitable technology for TCE and PCE elimination. Two oxidizing compounds have been specially suggested: $KMnO_4$ and Fenton's reagents [3,4]. However, there are some associated problems as its high pH dependence and the plug pore space due to the formation of Mn oxide as a reaction product in the case of the potassium permanganate oxidation [5].

Biodegradation is gaining momentum as an effective method for treating TCE and PCE polluted areas. Most of the TCE and PCE biodegradation demonstrations have been focused on reductive dechlorination by bacteria, which involves the sequential replacement of chlorine atoms on the alkene molecule by hydrogen atoms [6]. To date, only *Dehalococcoides ethenogenes* strain 195 has been known to degrade PCE to the non-toxic compound ethene, and the presence of *Dehalococcoides* group appears to be correlated with the complete dechlorination of chlorinated ethenes to ethane [7,8]. In any case, one of the main concerns of the implementing of this technology is the

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accumulation of less-chlorinated toxic compounds (such as *cis*-1,2-dichloroethylene) and carcinogenic intermediates (such as vinyl chloride).

Recently, it has been demonstrated the ability of the white-rot fungus *Trametes versicolor* to degrade aerobically both PCE and TCE [9,10]. Such studies were particularly interesting since constituted the first evidence of aerobic PCE degradation by fungi, which was considered non-biodegradable in presence of oxygen for many years. Additionally, a distinct advantage with the white-rot fungus *T. versicolor* in comparison to many of the bacterial systems is that the end-products obtained are far less toxic and can be readily degraded by other organism in the environment. Thus, these evidences open-up an interesting new area for bioremediation of PCE-contaminated environments.

This report presents the three-phase equilibriums of TCE and PCE in order to design a bioreactor to degrade aerobically these compounds with the fungus *T. versicolor*. The main requirements of this bioreactor are enough agitation, in order to obtain a good mass transfer and reach a fast equilibrium among the phases, and the vapour and liquid phase volume have to be enough to contain the necessary oxygen and biomass to degrade the pollutants, respectively. Besides, the bioreactor needs to be hermetically closed to avoid evaporation losses of these pollutants, because these contaminants are highly volatile. For this reason and due to these compounds can be adsorbed by the fungal biomass, it is necessary to study their distribution among the three phases to take it into account in the bioreactor design.

PCE and TCE are very volatile compounds at room temperature, so it is essential to know their distribution between the liquid and the gas phase at equilibrium. The parameter used to determine the distribution of a compound between the liquid and the gas phase is the Henry's constant [11].

Due to the great importance of this constant for these two compounds, an extensive bibliography research was done to obtain different values of Henry's constants at 25 °C, which is the experimental temperature. The PCE and TCE Henry's constants values are shown in Tables 1 and 2. Comparing these bibliographic values, few dispersion is observed among all the values for TCE and in the case of PCE, the values dispersion is greater than for TCE. Among all the values, the ones published in Ref. [12] have been used in this work, because they have been extensively referenced in the bibliography related to TCE and PCE degradation [13–17] and also, these values were determined by the EPICS method, which is one of the most used to determine Henry's constant values, but it was modified to increase its precision.

Table 1
TCE Henry's constant bibliographic values at 25 °C

Experimental method	Reference	H_c
Direct method	[18]	0.377
EPICS method in gas phase	[18]	0.457
EPICS method in liquid phase	[18]	0.369
EPICS_Spme method	[19]	0.415
Static methods	[20]	0.423
Static methods	[21]	0.420
Static methods	[22]	0.351
Batch air stripping	[23]	0.402
Calculated	[24]	0.459
Calculated	[25]	0.354
EPICS method	[26]	0.369
EPICS method modified	[11]	0.426
EPICS method modified	[12]	0.392

Table 2
PCE Henry's constant bibliographic values at 25 °C

Experimental method	Reference	H_c
Direct method	[18]	0.886
EPICS method in gas phase	[18]	1.032
EPICS method in liquid phase	[18]	0.647
Calculated	[25]	1.105
EPICS method	[26]	1.041
EPICS method	[20]	0.737
EPICS method modified	[11]	0.759
EPICS method modified	[12]	0.723

On the other hand, adsorption is a process where molecules of gases, liquids, or dissolved substances adhere to a solid or liquid surface. The adsorption isotherm is the equilibrium relation between the compound concentration in liquid or gas phase, which is expressed as mass concentration, and its concentration in solid or liquid particles, which is known as sorption capacity and it is expressed as compound adsorbed mass per adsorbent mass unit. Classical adsorption models, such as linear, Langmuir and Freundlich isotherms, are used to fit solid–liquid equilibrium [27].

2. Materials and methods

2.1. Fungal strains and chemicals

T. versicolor (ATCC#42530) was maintained by subculturing on 2% malt extract and 1.5% agar slants (pH 4.5) at 25 °C. Subcultures were made every 30 days. TCE and PCE were obtained from Sigma–Aldrich Co. (St. Louis, MO). Malt extract was obtained from Scharlau Co.

2.2. Culture methods

A mycelia suspension of *T. versicolor* was obtained by inoculation of four 1 cm^2 area agar plugs from the growing zone of fungus on malt agar (2%) to a 500 ml Erlenmeyer flask containing 150 ml of malt extract medium (2%) at pH 4.5. Flasks were incubated at 25 °C on an orbital shaker (135 rpm, $r = 25$ mm). After 4–5 days, the dense mycelia mass was suspended in an equal volume saline solution (0.8% NaCl) and was ground with a sterile X10/20 homogenizer (Ystral GmbH, Dottingen, Germany). This blended mycelia suspension was used as the inoculum. Pellets of *T. versicolor* were produced by using 1 ml of the mycelia suspension to inoculate 250 ml of malt extract medium in a 1 l Erlenmeyer flask. This was shaken (135 rpm, $r = 25$ mm) at 25 °C for 5–6 days. Subsequently pellets formed by this process were washed with a saline solution and were stored at 4 °C until use.

2.3. TCE and PCE adsorption experiments

All the experiments were performed with 20 ml serum bottles (real volume = 24 ml) sealed with teflon-coated grey butyl rubber stoppers (Wheaton, Millville, NJ) and aluminium crimps (Baxter Scientific Products, McGaw Park, Ill). Previously, a heat treatment at 120 °C during 30 min using an autoclave was used to eliminate *T. versicolor* metabolic activity and assure that there was no TCE or PCE degradation during the experiments.

The adsorption experiments were performed at different TCE or PCE initial concentrations (11 different initial concentrations between 5 and 20 mg/l for each one) and at every pollutant initial concentration, different pellets concentrations were performed (from 1 to 4 g/l (dry weight)). Each experimental combination was done in triplicate. First of all, the necessary pellets amount was added in each experimental bottle. Then, 19 ml of distilled water and 3 ml of sodium azide solution (1%), which was used to avoid contamination problems, were added to each inoculated bottle to assure that gas phase in each bottle was minimal. Then, 44 μl from a TCE or PCE concentrated solution in ethanol was added to each inoculated bottle by means of a pressure-lok gas-tight syringe (VICI Precision Sampling, Baton Rouge, LA) through the stoppers. Afterwards the bottles were incubated at 25 °C on an orbital shaker (190 rpm, $r = 25$ mm) during 24 h, which is enough time to reach the adsorption equilibrium, in an inverted position to minimize gas leakage. Afterwards, pellets dry weight was measured for each experimental bottle. Each experiment included un-inoculated controls to determine PCE or TCE initial concentration in experimental bottles.

The sorption capacity of TCE or PCE can be calculated based on the balance where

$$q_i = \frac{V(C_0 - C_{\mathrm{liq},i})}{m_{\mathrm{biom},i}} \quad (1)$$

where q_i is the adsorbed mass per biomass dry weight unit (mg g^{-1}); V is the solution volume (l); C_0 is the initial concentration in liquid phase (mg l^{-1}), obtained from un-inoculated controls; $C_{\mathrm{liq},i}$ is the compound concentration in liquid phase at equilibrium conditions (mg l^{-1}); $m_{\mathrm{biom},i}$ is biomass dry weight (g).

2.4. TCE and PCE desorption experiments

These experiments were performed at a TCE or PCE initial concentration of 20 mg/l, the pellets concentration was 2.7 g/l (dry weight) and it was done in triplicate.

The adsorption experimental process was equal to the one detailed previously. After adsorption process was finished, each inoculated bottle was opened and pellets contained in each bottle were transferred to another 20 ml serum bottle. Then, 19 ml of distilled water and 3 ml of sodium azide solution (1%) were added to each bottle. Afterwards the bottles were incubated at 25 °C on an orbital shaker (190 rpm, $r = 25$ mm) during 24 h. Finally, pellets dry weight was measured for each experimental bottle.

2.5. TCE and PCE degradation experiments

All the experiments were performed using 125-ml serum bottles sealed with teflon-coated grey butyl rubber stoppers (Wheaton, Millville, NJ) and aluminium crimps (Baxter Scientific Products, McGaw Park, Ill). Each bottle was inoculated with 0.04 g (dry weight) of pellet of *T. versicolor*. A volume of 10 ml of defined medium [10] was added to each inoculated bottle and subsequently was oxygenated for 1 min and sealed immediately. Then, 20 μl of a solution of PCE or TCE in ethanol was added by means of a pressure-lok gas-tight syringe (VICI Precision Sampling, Baton Rouge, LA) through the stoppers to give the desired TCE or PCE concentration in the liquid media. The bottles were shaken vigorously for 30 min in an inverted position (to minimize gas leakage) and subsequently were incubated at 25 °C on an orbital shaker (135 rpm, $r = 25$ mm), also in an inverted position. In those cases where reoxygenation took place, 5 ml of pure oxygen was added by means of a pressure-lok gas-tight syringe through the stoppers.

Each experiment included un-inoculated and heat-killed controls. Heat-killed controls consisted of autoclaved cultures that had been pre-grown for 7 days under conditions identical to those of the experimental cultures. Degradation yield at a specified interval was calculated by comparing concentration in the un-inoculated blanks with those in the experimental bottles. All degradation values were corrected for the sorption values determined using the heat-killed controls. PCE or TCE concentration values were also corrected considering the water volume added with pellets. Each bottle was sacrificed at each time point for analysis.

2.6. PCE and TCE analysis

The concentration of PCE or TCE was determined by static headspace gas chromatography. All samples were equilibrated at 25 °C before analysis. A 1 ml liquid sample from each experimental bottle was transferred to 4 ml sodium azide solution (1%) in a 10 ml vial and sealed immediately with a teflon-coated stopper. The vial was placed in a headspace sampler Agilent 7964 (Agilent Technologies, Palo Alto, CA) and was heated to 85 °C for 50 min to volatilize all the pollutant contained in the liquid phase. Subsequently, a 1-ml headspace sample was injected automatically into a gas chromatograph (Agilent 6890N) equipped with a column Agilent HP-5 ($30 \times 0.32 \times 0.25$) and a flame ionization detector.

The GC operating conditions were as follows: column temperature, 40 °C (2 min), slope 4 °C/min, 50 °C (1 min), slope 10 °C/min, final temperature: 160 °C; injector temperature, 125 °C; flame ionization detector temperature, 260 °C; carrier gas He at 7 psi pressure. Data was acquired and quantified by Millennium 32 software (Waters, Milford, MA).

PCE or TCE concentration in liquid media in each experimental bottle was determined by comparing peak areas obtained with those of external standards. After that, pollutant concentration in gas phase was calculated by using Henry's law constants at experimental temperature (25 °C) chosen among the biblio-

graphic values. The constant values used were 0.392 for TCE and 0.723 for PCE [12].

2.7. Pellets dry weight

Pellets dry weight was determined by vacuum filtering the cultures with glass filters weighted previously (Whatman GF/C, 47 mm diameter). The filters containing the biomass were placed in glass dishes and dried at 105 °C to constant weight.

2.8. Pellets density

Pellets density was determined by weighing a fixed pellets wet weight and it was added to a graduated cylinder with a fixed water volume. The displaced water volume by pellets is used to calculate the pellets density. Afterwards, dry weight of these pellets was carried out to calculate pellets density.

3. Results and discussion

Adsorption of water pollutants by different kind of biomass has been demonstrated to be important in many cases [28–30]. In the case of *T. versicolor*, the adsorption of different sort of dyes has been shown to be significant as well [31,32]. So it is important to assess TCE and PCE adsorbed mass by *T. versicolor* to evaluate their degradation.

On the other hand, the obtaining of PCE and TCE adsorption isotherms is very useful for the design of a three-phase bioreactor to degrade these compounds in order to quantify their degradation and to distinguish it from their elimination.

3.1. Adsorption isotherms of TCE and PCE on T. versicolor in the form of pellets

Experiments to obtain adsorption isotherms were carried out with experimental bottles completely full of liquid and biomass to minimize pollutants volatilization to gas phase. A great variety of TCE or PCE initial concentrations and pellets concentrations were used, because an elevated number of experimental values were necessary to obtain reliable isotherms parameters, due to their high volatility and the low initial concentrations used which can provoke an important experimental error.

Figs. 1 and 2 show the adsorption experimental values and the fittings of linear adsorption isotherm to these values. The values of the parameters of linear isotherm and its correlation coefficients (r^2) are listed in Table 3. Due to the low TCE and PCE concentration range studied, the experimental data of the adsorption according to Langmuir and Freundlich isotherms were located in the linear zone. So the linear isotherm is enough to describe the adsorption of these pollutants by biomass.

Table 3
Fitting constants and coefficients of correlation (r^2) for the fitting of the linear isotherm to the experimental data for TCE and PCE adsorption by pellets

TCE	PCE
$K = 0.110$	$K = 0.176$
$r^2 = 0.76$	$r^2 = 0.84$

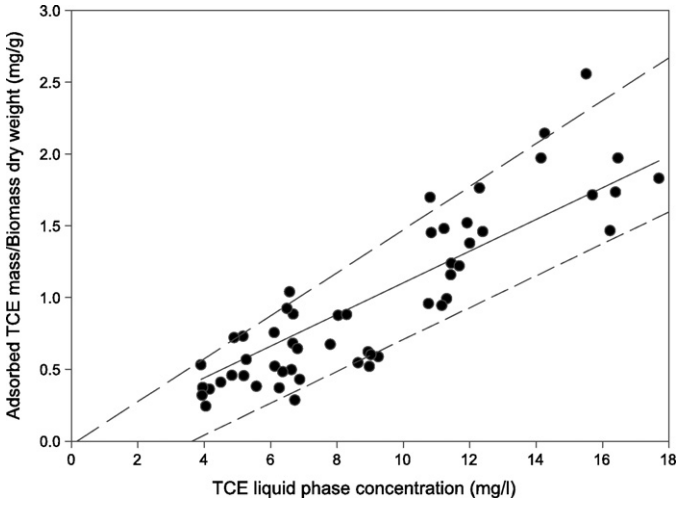

Fig. 1. TCE experimental values and fitting for linear isotherm. (●) Corresponds to adsorption experimental values, the continuous line corresponds to the linear regression of experimental values and the discontinuous lines correspond to the top and lower confidence interval at 95%.

From these figures it can be observed that linear isotherm fits rather well to adsorption experimental values for TCE as well as for PCE. Furthermore, a statistical study was done and the top and lower confidence intervals at 95% are represented in Figs. 1 and 2. These intervals mean that there is a certainty of 95% to found an experimental value between them and is used to detect which values move away from the general tendency, provoking some dispersion to regression equation results.

From the confidence intervals of the figures, it can be observed that linear isotherm have most of the experimental values between the confidence intervals and that means there is not a high scattering of the experimental values. For this reason and

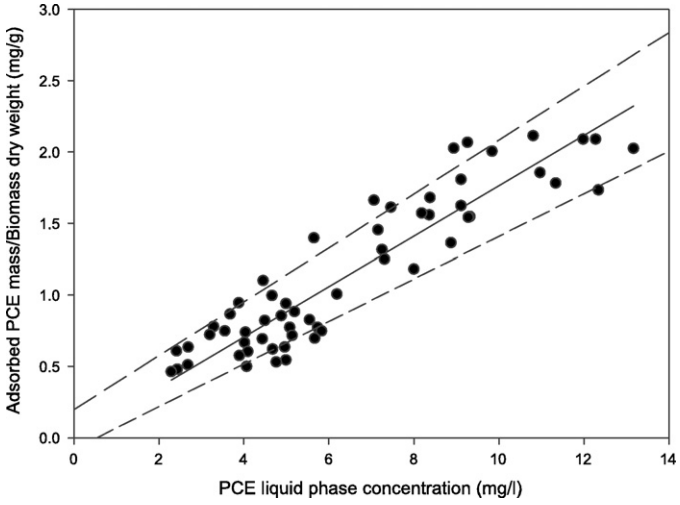

Fig. 2. PCE experimental values and fitting for linear isotherm. (●) Corresponds to adsorption experimental values, the continuous line corresponds to the linear regression of experimental values and the discontinuous lines correspond to the top and lower confidence interval at 95%.

Table 4
TCE distribution among different phases at equilibrium in experimental bottles at initial conditions

C_0 (liquid phase) (mg/l)	C (liquid phase) (mg/l)	C (gas phase) (mg/l)	C (solid phase) (mg/l)	% adsorbed to biomass
5	4.36	1.71	11.35	12.8
10	8.72	3.42	22.70	12.8
15	13.08	5.13	34.05	12.8
20	17.44	6.84	45.40	12.8

according to regression coefficients obtained, linear regression is suitable to describe TCE and PCE adsorption by the fungus.

On the other hand, TCE and PCE desorption has been studied and it has been checked that their adsorption is reversible and that means that during degradation process, TCE and PCE adsorption not only happens initially, but there is an equilibrium between pollutants adsorbed mass and their concentration in liquid phase during all degradation process. Thus, it is necessary to know the compound concentration at liquid phase during all the degradation process to obtain the compound adsorbed mass at equilibrium. However, if the adsorption was irreversible, the adsorbed mass at equilibrium would be calculated using only the initial conditions.

3.2. Evaluation of TCE and PCE linear isotherm in a three-phase reactor in degradation conditions

This section shows the application of TCE and PCE linear isotherm to calculate their distribution in a three-phase bioreactor used to degrade them by *T. versicolor* at the initial conditions. Besides, a comparison between TCE and PCE experimental and calculated degradation yields is shown in this section in order to demonstrate that linear isotherm can be used to describe the adsorption of these two compounds by biomass in the degradation experimental conditions.

All degradation experiments were realized with a reactor with a ratio between gas phase and liquid phase volume of 14.7. This ratio is very high because *T. versicolor* degrade aerobically TCE and PCE and it is necessary a large gas phase volume to contain an elevated oxygen amount, which was added before degradation because during the process the reactor was hermetically closed to avoid evaporation losses.

Moreover, it was necessary to determine experimentally the pellets density (23.58 g/l) to know the relation between their volume and their dry weight. 0.09 g of pellets (dry weight) was used in the reactor, that it is equivalent to a ratio between biomass volume and liquid volume of 0.4, using the pellets density determined experimentally. The distribution of TCE or PCE at equilibrium was done for four different initial concentrations in liquid phase (5, 10, 15 and 20 mg/l). Using TCE and PCE Henry's constants, the initial mass in gas and liquid phase was calculated for each initial concentration. Then a total mass balance at equilibrium was done

$$m_0 = m_L + m_G + m_S \tag{2}$$

where m_0 is TCE or PCE initial mass added at experimental bottles and m_L, m_G and m_S are TCE or PCE mass in liquid phase, gas phase and solid phase, respectively, at equilibrium.

To solve Eq. (2) m_G and m_S are isolated in regard to m_L, using TCE and PCE Henry's constants and linear isotherm

$$m_G = H_c m_L \frac{V_G}{V_L} \tag{3}$$

$$m_S = KX\left(\frac{m_L}{V_L}\right) \tag{4}$$

where H_c is TCE or PCE dimensionless Henry's constant, V_G is gas phase volume, V_L is liquid phase volume, K is the linear isotherm parameter and X is pellets dry weight.

Results obtained for each TCE and PCE initial concentration in liquid phase are shown in Tables 4 and 5. These results demonstrate that adsorption of these two compounds by the pellets is not very high, but it is significant (12.8% for TCE and 12.0% for PCE) and this means that it is an important parameter to take into account in the bioreactor design. Tables 4 and 5 show that as initial total mass increases, logically TCE and PCE adsorbed mass increases too. Also, it can be observed that the adsorption percentage is very similar in both cases and the percentage is a little higher for TCE than for PCE due to its less volatility.

In order to validate the linear isotherm in degradation conditions, TCE and PCE experimental and calculated degradation yields were obtained and they are shown in Tables 6 and 7. The experimental degradation yields were obtained comparing TCE and PCE concentration in un-inoculated blanks with the concentration in inoculated bottles. Also, the adsorbed mass values were obtained experimentally in each case and they were used to correct the degradation values. The calculated degradation yields were obtained with the same experimental values used to

Table 5
PCE distribution among different phases at equilibrium in experimental bottles at initial conditions

C_0 (liquid phase) (mg/l)	C (liquid phase) (mg/l)	C (gas phase) (mg/l)	C (solid phase) (mg/l)	% adsorbed to biomass
5	4.40	3.18	18.30	12.0
10	8.81	6.36	36.60	12.0
15	13.20	9.54	54.90	12.0
20	17.60	12.72	73.21	12.0

Table 6
Comparison between TCE experimental and calculated degradation yields

C_0 in liquid phase (mg/l)	% experimental degradation	% calculated degradation	% error
5	74.8	74.0	1.0
10	66.1	61.2	7.4
15	56.7	52.6	7.2
20	55.6	40.0	28.1

Table 7
Comparison between PCE experimental and calculated degradation yields

C_0 in liquid phase (mg/l)	% experimental degradation	% calculated degradation	% error
3	36.9	37.1	0.6
5	26.4	20.3	22.9
10	15.2	14.3	6.0

obtain the experimental degradation yields, excepting the TCE and PCE adsorbed mass, which was calculated using the linear isotherm.

Tables 6 and 7 show that for most TCE and PCE initial concentrations, experimental and calculated degradation yields are very similar and in the cases where the error is higher (about 28% for TCE and 23% for PCE), the cause is that adsorbed mass values obtained experimentally are very low compared with other values obtained in the same experimental conditions, because the possible experimental uncertainty in a single adsorption determination can cause a higher variation in the degradation yield than using the adsorption value obtained from adsorption isotherm. From this comparison between experimental and calculated degradation yields, it can be stated that linear isotherm describes correctly the adsorption of TCE and PCE by *T. versicolor* in the form of pellets. As a consequence, it is not necessary to obtain experimentally the adsorbed mass in each TCE or PCE degradation experiment in a three-phase reactor.

4. Conclusion

The equations that describe TCE and PCE distribution among the three phases at equilibrium have been determined or bibliographically obtained and it has been checked that they can be used in degradation conditions. Thus, these equations can be used in a three-phase bioreactor design to degrade TCE and PCE with the white-rot fungus *T. versicolor* in the form of pellets.

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