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Kinetic characterization of toluene biodegradation by *Rhodococcus erythropolis*: Towards a rationale for microflora enhancement in bioreactors devoted to air treatment

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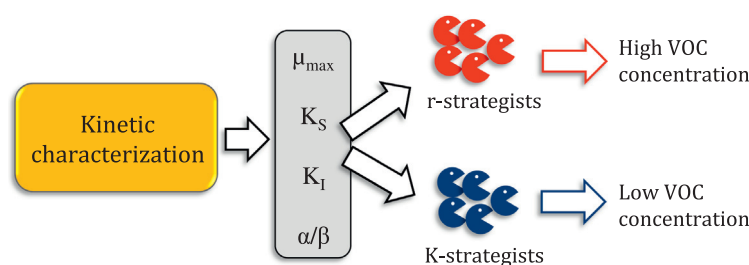
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HIGHLIGHTS

- The kinetic characterization of *Rhodococcus erythropolis* was performed using toluene as a model VOC.
- The Luedeking–Piret correlation described accurately the CO₂ production.
- *R. erythropolis* can be used for boosting bioreactors treating low toluene concentrations.
- Toluene mineralization was mainly related to biomass growth.

GRAPHICAL ABSTRACT



Keywords:

Biological air treatment
Kinetic characterization
Rhodococcus erythropolis
Toluene
Volatile organic compounds
Mathematical modelling

ABSTRACT

The kinetic characterization of microorganisms degrading volatile organic compounds (VOCs) is difficult due to the low substrate concentration prevailing in these processes. In this work, a simple, accurate and easy-to-implement methodology was proposed to perform the kinetic characterization of VOC-degrading microorganisms. The methodology was applied to *Rhodococcus erythropolis* using toluene as a model VOC. Besides the assessment of traditional kinetic parameters such as the maximum specific growth rate (μ_{\max}), substrate half-saturation (K_S) and substrate inhibition (K_I), the methodology here proposed yielded the α/β kinetic parameter, which indicates the relative relevance of the biomass growth rate and bacterial density on the VOC mineralization performance. *R. erythropolis* exhibited K_S , K_I and μ_{\max} values of 0.39 g m⁻³, 54 g m⁻³ and 0.21 h⁻¹, respectively. The potential of this microorganism (supporting extremely low K_S and high K_I values) for boosting the performance of bioreactors treating low toluene concentrations was highlighted. Furthermore, α/β ratios of ~50 to ~500 were recorded at initial toluene gas concentrations of 1.5–5.2 g m⁻³, respectively. These α/β ratios indicated that toluene mineralization under the working conditions was mainly related to the biomass growth rather than to the biomass concentration.

1. Introduction

The removal of volatile organic compounds (VOCs) from contaminated airstreams has become a major public concern.

Nowadays, VOC emissions are subject to severe environmental constraints because of their negative impact on human health and the environment [1,2]. Among the available technologies for air pollution control, biological technologies in many cases constitute the most cost-effective technology for VOC removal [3]. VOC biodegradation is usually the result of many metabolic processes performed by a stratified microbial community along a packed

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Nomenclature

$\text{CO}_{2\text{G}}$	CO_2 concentration in gas phase (g m^{-3})
$\text{CO}_{2\text{L}}$	CO_2 concentration in liquid phase (g m^{-3})
H_{CO_2}	dimensionless Henry's law constant for CO_2
K_I	toluene inhibition constant from the Haldane–Andrews model (g m^{-3})
K_S	toluene half-saturation constant (g m^{-3})
S	toluene concentration in liquid phase (g m^{-3})
X	biomass concentration (g m^{-3})

$Y_{X/S}$	biomass-to-toluene yield (gX gS^{-1})
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Greek letters

α	constant of the Luedeking–Piret model ($\text{gCO}_2 \text{gX}^{-1}$)
β	constant of the Luedeking–Piret model ($\text{gCO}_2 \text{gX}^{-1} \text{h}^{-1}$)
μ	specific biomass growth rate (h^{-1})
μ_{max}	maximum specific biomass growth rate (h^{-1})

bed as occurs in biofilters and biotrickling filters. Therefore, bioreactors devoted to air pollution control are constituted by complex and structured ecosystems [4,5].

The VOC concentration in the polluted airstream is a very important operating variable since it might determine the resulting microbial community established in the biofilter [6]. From a microbial ecology point of view, the microorganisms are classified according to their competitive abilities to survive [7]. Therefore, it is convenient to consider two main kinds of microorganisms: *K*- and *r*-strategists. *K*-strategist microorganisms grow slow but use efficiently the substrate under limiting conditions (microorganisms with low K_S and low μ_{max} values). On the contrary, *r*-strategists grow quickly under non-limiting substrate conditions (microorganisms with high K_S and μ_{max} values). As a matter of fact, several investigations revealed a predominant role of *K*-strategist microorganisms such as *Rhodococcus* or *Pseudonocardia* strains in biofilters operated with VOC concentrations $\leq 1.25 \text{ g m}^{-3}$ [8–10], while *r*-strategists such as *Pseudomonas* strains have been reported to play a predominant role in biofilters operated with VOC concentrations $\geq 1.8 \text{ g m}^{-3}$ [11,12]. Considering the available experimental data, it is difficult to establish a general VOC concentration boundary at which *K*- or *r*-strategists become predominant. However, the empirical evidences so far reported certainly encourage VOC removal enhancement by means of the rational selection of the type of microorganisms (*K*- or *r*-strategists) depending on the inlet VOC concentration. For this purpose, a comprehensive kinetic characterization of VOC-degrading microorganisms is therefore necessary.

The key objectives and hypotheses of the work are: (i) to perform the kinetic characterization of *Rhodococcus erythropolis* isolated from a biofilter and using toluene as a model VOC; (ii) to describe toluene oxidation by *R. erythropolis* by using a mathematical modelling approach incorporating the Luedeking–Piret correlation and (iii) to obtain from the mathematical model insightful information regarding the relevance of the biomass growth and the biomass concentration on the VOC mineralization.

2. Theoretical framework

The microbial kinetic characterization was done by means of the Haldane–Andrews model:

$$\mu = \mu_{\text{max}} \frac{S}{K_S + S + S^2/K_I} \quad (1)$$

This kinetic model was used since toluene is a toxic compound able to inhibit the microbial growth. Moreover, the assessment of K_I was useful to perform comparisons on the toluene tolerance of *R. erythropolis* compared to that reported for other microorganisms. The CO_2 production data recorded at several initial toluene concentrations were modelled by means of the Luedeking–Piret correlation [13]:

$$\frac{d\text{CO}_{2\text{L}}}{dt} = \alpha \frac{dX}{dt} + \beta X \quad (2)$$

where α and β are constants accounting for the CO_2 production related to the biomass growth and the biomass concentration, respectively. Therefore, changes in the α/β ratio under varied VOC concentrations provide relevant information on the VOC oxidation mechanisms. The CO_2 concentration in gas phase was obtained from Eq. (3) using the dimensionless Henry's law constant ($H_{\text{CO}_2} = 1.17$):

$$\frac{d\text{CO}_{2\text{G}}}{dt} = \frac{d\text{CO}_{2\text{L}}}{dt} H_{\text{CO}_2} \quad (3)$$

Finally, changes in the toluene and biomass concentrations in time were described by means of Eqs. (4) and (5):

$$\frac{dS}{dt} = -\frac{\mu}{Y_{X/S}} X \quad (4)$$

$$\frac{dX}{dt} = \mu X \quad (5)$$

The model represented by Eqs. (2)–(5) is a simplified description of the system and allows for the characterization of the toluene mineralization coupled to the biomass concentration and biomass growth rate. The following assumptions were considered: (i) the liquid phase was perfectly mixed and thus no mass transfer limitation occurred, (ii) the Henry's law constant remains constant throughout the experimental system and therefore a precise estimation of the toluene and CO_2 concentrations in liquid phase can be done from the gas phase concentrations. K_S and K_I were obtained from Eq. (1) by plotting the initial μ vs the initial toluene concentrations and performing a nonlinear regression (Microsoft Solver® 2010). The maximum initial growth rate experimentally determined was considered as μ_{max} . Eqs. (2)–(5) were solved simultaneously using ModelMaker® software (Cherwell Scientific, UK). The Runge–Kutta method was used for solving the set of differential equations, whereas α and β were fitted using the Levenberg–Marquardt method.

3. Material and methods

3.1. Bacterial strain

R. erythropolis was isolated from a biofilter treating a mixture of eleven VOCs (including toluene) as described by Khammar et al. [5]. The identification of the strain was confirmed by 16S rDNA sequence analysis: a suspension of *R. erythropolis* strain in 100 μL of sterile water was placed in a 1.5-mL tube. The kit NucléoSpin Tissue (Macherey–Nagel, Hoerd, France) was used for DNA extraction. The SSU rDNA was then amplified by PCR using the rDNA universal primer W002 (5'GNTACCTTGTTACGACTT3', position R1492) with the rDNA bacteria primer W018 (5'GAG-TTTGATCMTGGCTCAG3', position F9) for 16S rDNA amplification.

DNA products were then sequenced (Millegen, Toulouse, France). A sequence of 1500 bp was carried out and used for comparison with sequences available in a database (Genbank). In order to confirm the sequence analysis result, the primer sequences Re1 (5'CGTCTAATACCGGATATGACCTCTATC3', position R165) and Re2 (5'GCAAGCTAGCAGTTGAGCTGCTGGT3', position F633) were used [14]. Finally, a sequence of 449 bp was carried out.

3.2. Toluene biodegradation tests

The kinetic characterization of the *R. erythropolis* strain was performed using several toluene concentrations under sterile conditions in batch experiments. The batch tests were performed in 500-mL flasks containing 250 mL of mineral salt medium prepared according to Juteau et al. [8] and sealed with a Teflon-coated cap (Interchrom, Montluçon, France). The flasks were connected to a HP5890 gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) equipped with flame ionization and thermal conductivity detectors (FID and TCD, respectively) via sterile 1/16" Teflon-tubes (Fig. 1). Toluene (99% purity, Carbo Erba, Italy) was used as the sole carbon and energy source. Initial toluene concentrations in the gas phase ranged from 0.1 to 10 g m⁻³. Toluene equilibration between the liquid and gas phases was allowed for 24 h and then the flasks were inoculated with a biomass concentration of approximately 0.8 g m⁻³. The biodegradation tests were carried out at 28 °C and a magnetic agitation speed of 200 rpm.

In those tests performed under toluene gas concentrations relevant for biological air pollution treatment (1–5 g m⁻³, Delhoménie et al. [15]), the final biomass concentration was also determined in order to assess the $Y_{X/S}$ values. This allowed the use of Eqs. (2)–(5) to get insights on the toluene mineralization mechanisms by means of determining the α/β ratio.

3.3. Biomass quantification

Determination of the biomass concentration at the beginning and the end of the experiments was performed as follows: 1-mL of the inoculated bacterial suspension and 50-mL of the liquid phase from the bottle tests were centrifuged for 5 min at 10,000g (Allegra 64R Centrifuge, Beckman Coulter Inc., Brea, CA, USA).

The supernatant was discarded and the biomass pellet was resuspended in mineral salt medium. Thereafter, the resuspended cell suspension was sonicated for 3 min (Branson, Danbury, CT, USA) at an energy output of 40 W. The protein concentration was then determined according to the Quick Start™ Bradford Protein Assay (Bio-Rad, Hercules, CA, USA). The biomass concentration was obtained assuming a protein content of 0.55 g per gram of bacterial biomass according to Villadsen et al. [16].

3.4. Analytical methods

The gas chromatograph was equipped with two automatic gas sampling valves (250–500 µL) arranged in series. Each gas sampling valve was connected to a chromatographic column and a specific detector (FID for toluene and TCD for CO₂). Toluene and CO₂ measurements in gas phase were performed every 22 min. The oven temperature was maintained at 40 °C for 5 min and increased up to 70 °C at a rate of 10 °C min⁻¹. The temperature was then maintained at 70 °C for 8 min before decreasing to 40 °C at a rate of 10 °C min⁻¹. This temperature was maintained for 4 min and the cycle was repeated.

For toluene quantification, a 30-m HP-1 capillary column (Hewlett Packard, Palo Alto, CA, USA) was used. Helium was the carrier gas at a flow rate of 15 mL min⁻¹. The FID temperature was kept at 220 °C. For CO₂ quantification, a 1.5-m Chromosorb 101 packed column (Restek Corporation, Bellefonte, PA, USA) was used. Helium was the carrier gas at a flow rate of 23.5 mL min⁻¹. The TCD temperature was kept at 250 °C. Calibration curves for toluene and CO₂ were prepared with external standards for determining the gaseous concentrations. For all the batch tests carried out, the relative standard deviation of gaseous measurements showed a variation between 0.6% and 2.4%.

4. Results and discussion

4.1. Microbial kinetic characterization

Before performing the kinetic characterization, a 16S rDNA sequence analysis was done in order to confirm the identity of the isolated *Rhodococcus* strain. The amplification of partial 16SrDNA

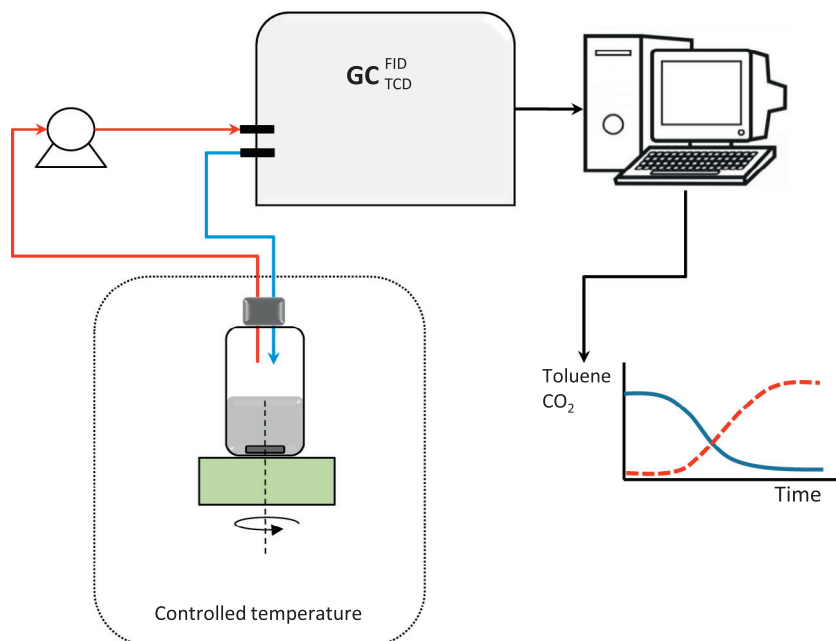


Fig. 1. Schematic representation of the setup used for the toluene biodegradation experiments.

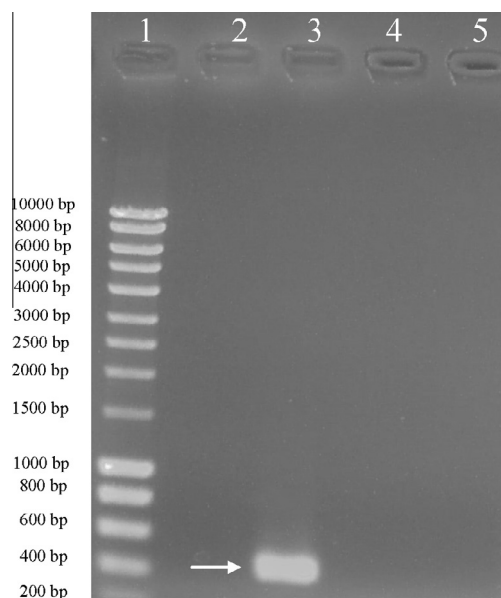


Fig. 2. Amplification of partial 16SrDNA extracted from the *Rhodococcus erythropolis* strain. (1) Smart ladder marker, (2) negative control, (3) amplified 16SrDNA with specific primers Re1 and Re2, (4) negative control of amplified 16SrDNA with Re1, and (5) negative control of amplified 16SrDNA with Re2.

with specific primers (Fig. 2) confirmed that the isolated bacterium was a *R. erythropolis*-like microorganism (accession number DQ518913).

Thereafter, biodegradation tests were performed with toluene gas concentrations ranging from 0.1 to 10 g m⁻³ (corresponding to toluene liquid concentrations of 0.33–33 g m⁻³). The Haldane–Andrews model fitted accurately the data obtained from the batch tests (r^2 of 74%), allowing for K_S and K_I determination (Fig. 3). It is important to stress that this *Rhodococcus* strain was isolated from the bottom of a peat biofilter fed with a complex mixture of VOCs in a downward flow mode [5]. Aromatic VOCs such as toluene, ethylbenzene and *p*-xylene were mainly removed in the bottom of the biofilter. The microbial community established at the bottom of the biofilter was adapted to a resource-restricted environment due to the low VOC concentration recorded in that particular zone (~0.02–0.03 g m⁻³). Such conditions were then suitable for the proliferation of *K*-strategists and it was not surprising to isolate a microbial strain belonging to the *Rhodococcus* genus [8].

The kinetic parameters assessed for *R. erythropolis* together with a compilation of kinetic data reported for other toluene-degrading microorganisms are listed in Table 1. It is worth noting that

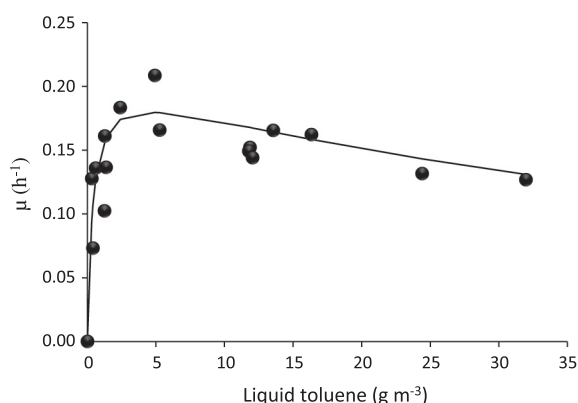


Fig. 3. Initial growth rates vs initial toluene concentrations. The continuous line represents the Haldane–Andrews model fitting.

R. erythropolis exhibited the lowest K_S value, which was up to 160 times lower than that reported for *Pseudomonas* strains. In agreement with the characteristics of *K*-strategist microorganisms, *R. erythropolis* exhibited a relatively low μ_{\max} value, which was in the worst case 7.4 times lower relative to the μ_{\max} supported by *Pseudomonas* strains. It must be remarked that microorganisms belonging to the *Pseudomonas* genus have been identified as *r*-strategists and therefore they supported the highest μ_{\max} values [8]. Table 1 also includes μ_{\max} values lower than those determined for *R. erythropolis* [17,18]. However, such studies were performed under extremely high toluene gas concentrations (121 and 150 g m⁻³) and thus, these microorganisms were likely grown under strong inhibiting conditions. Surprisingly, the K_I value exhibited by *R. erythropolis* was similar to those reported for some *Pseudomonas* strains [15,16], highlighting the fact that this *Rhodococcus* strain can tolerate equivalent toluene concentrations compared to some *r*-strategists.

The kinetic characteristics of the *Rhodococcus* strain here studied were very interesting from an optimization point of view. Since this microorganism exhibits an extremely low K_S , it can be included in the inoculum to boost the performance of bioreactors treating low concentrations of aromatic compounds. This would allow for an improved VOC removal and shorter start-ups. Another key feature of *R. erythropolis* was its relatively high K_I value, which can be very important in shock load episodes. During shock loads, the VOC-mediated toxicity is species-selective and therefore the presence of microorganisms with high K_I values could favour the biofilter recovery after disturbance [4,22]. Our study revealed that the use of *K*-strategists such as *R. erythropolis* might result in improved process robustness.

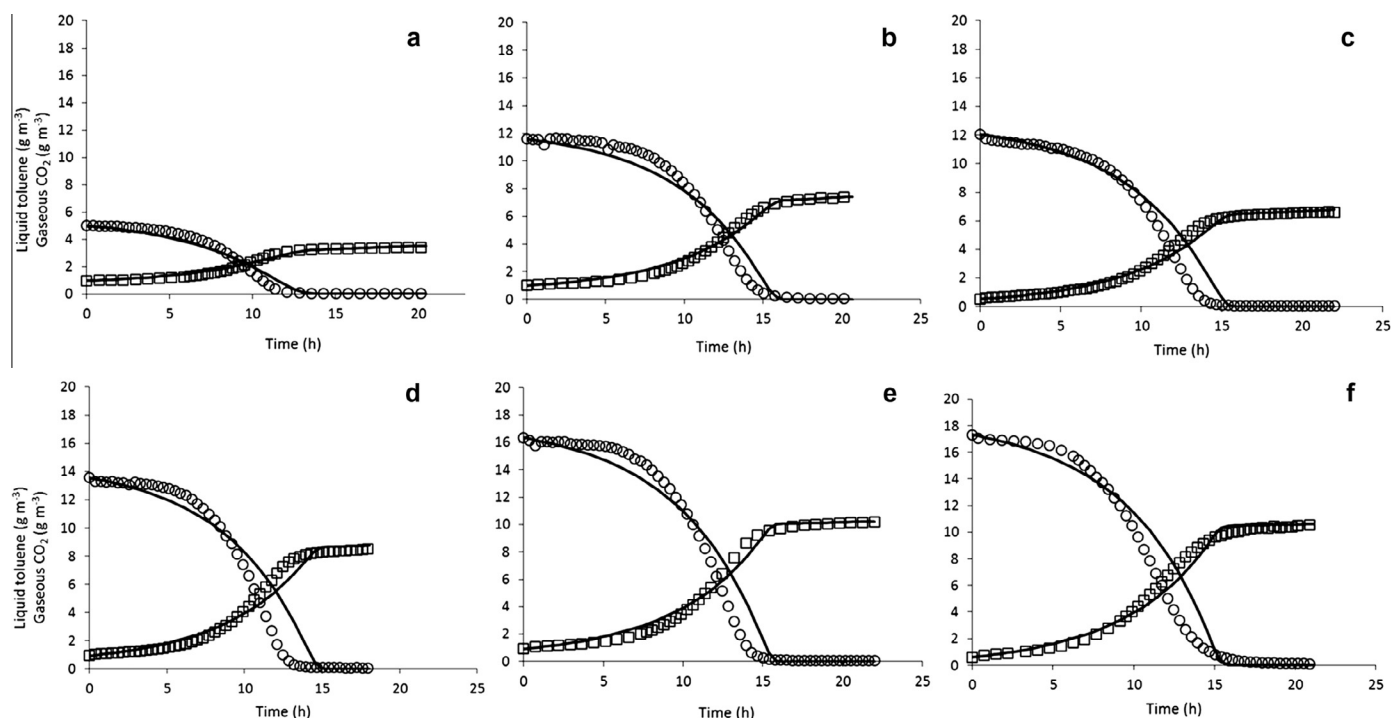
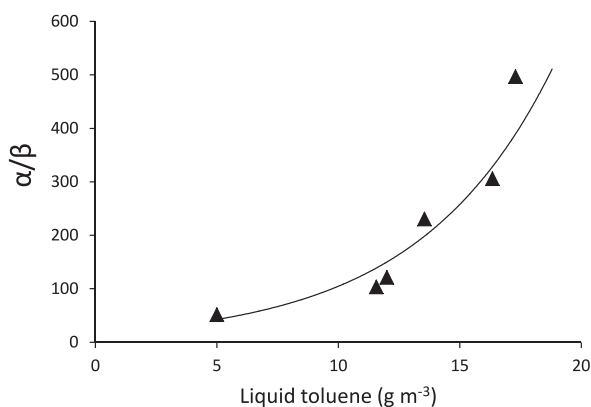
4.2. Modelling of toluene mineralization

A mathematical approach incorporating the Luedeking–Piret correlation (Eq. (2)) was used to describe the toluene biodegradation and CO₂ production under conditions relevant for biological air treatment. The toluene gas concentrations considered for this mathematical analysis ranged between 1.5 and 5.2 g m⁻³, corresponding to liquid concentrations of 5.0–17.3 g m⁻³. The proposed model fitted well the overall set of experimental data, being particularly accurate for describing the CO₂ production profiles (Fig. 4). An overall correlation coefficient (r^2) of 96% between the model results and the experimental data was achieved. A very useful feature of the Luedeking–Piret correlation is that it relates the CO₂ production with both the biomass growth and biomass concentration, the α and β values weighting each of these two aspects on the CO₂ production.

It was observed that the initial toluene concentration indeed affected the mineralization mechanisms of *R. erythropolis* since the $Y_{X/S}$ values decreased as the initial toluene concentration increased. Thus, $Y_{X/S}$ values of 0.98, 0.72, 0.63, 0.49, 0.45 and 0.40 g g⁻¹ were recorded for initial toluene concentrations in liquid phase of 5.0, 11.6, 12.0, 13.5, 16.3 and 17.3 g m⁻³, respectively. These results clearly showed that less biomass was produced per gram of toluene degraded as the VOC concentration increased. On the other hand, the α/β ratio increased exponentially with the initial toluene concentration (Fig. 5). Such changes in the α/β ratio indicated that the CO₂ production was mainly related to the biomass growth rather than to the biomass concentration. Interestingly, α/β ratios of ~50 and ~500 were recorded at initial toluene concentrations of 5.0 and 17.3 g m⁻³ in liquid phase, respectively. This indicates that despite playing a small role, the biomass concentration was relevant in some extent in the mineralization performance at the lowest toluene concentration tested. However, the CO₂ production became overwhelmingly related to the biomass growth at liquid toluene concentrations higher than 5 g m⁻³.

Table 1Parameters of the Haldane–Andrews model for *Rhodococcus erythropolis* and other toluene-degrading microorganisms reported in the literature.

Microorganism	μ_{\max} (h ⁻¹)	K_S (g m ⁻³)	K_I (g m ⁻³)	Maximum C_G value (g m ⁻³)	Reference
<i>Pseudomonas putida</i>	0.17	62.56	380	121	[17]
Activated sludge	0.11	56.60	29	150	[18]
<i>Pseudomonas putida</i> 54G	0.42	3.98	43	15	[19]
Bacterial consortium	1.50	11.03	79	21	[20]
<i>Pseudomonas putida</i> OI	1.56	15.07	44	21	[20]
<i>Pseudomonas putida</i> F1	0.78	5.00	753	55	[21]
<i>Rhodococcus erythropolis</i>	0.21	0.39	54	10	Present study

**Fig. 4.** Time courses of toluene concentration in liquid phase (circles) and CO₂ concentration in gas phase (squares) during the biodegradation experiments at initial toluene concentrations of: (a) 5.0 g m⁻³, (b) 11.6 g m⁻³, (c) 12.0 g m⁻³, (d) 13.5 g m⁻³, (e) 16.3 g m⁻³ and (f) 17.3 g m⁻³. Continuous lines represent the model fitting (Eqs. (2)–(5)).**Fig. 5.** Effect of the initial toluene concentration in liquid phase on the α/β ratio.

The α/β ratios here obtained revealed that toluene mineralization in biological air treatment systems can be boosted if the biomass growth is promoted, while a high biomass concentration does not guarantee a good toluene mineralization performance. From a practical point of view, the biomass growth can be

promoted by constantly removing a biomass fraction from the bioreactor, allowing for the continuous growth of new cells. In the case of biotrickling filters and liquid-based bioreactors (e.g. bubble columns, airlifts and two-phase partitioning bioreactors), biomass can be washed out by means of a constant aqueous phase renewal. In fact, several studies on VOC removal in biotrickling filters and liquid-based bioreactors stressed the relevance of the liquid phase renewal in order to obtain high and stable VOC abatement performance [23–25]. In the case of biofilters, the mineral salt medium irrigation besides being necessary to provide nutrients and moisture it can also be helpful for washing out old biomass and stimulate the growth of new cells. In a recent study, Lebrero et al. [26] showed the key role of the liquid irrigation frequency on stable hexane abatement in a compost biofilter.

It is important to stress that the modelling approach used in the present work was relatively simple and easy to implement. Therefore, the α/β ratio can be used as a tool to obtain information on the relative relevance of the biomass growth and the biomass concentration on the VOC mineralization performance. Thus, for a given VOC concentration the α/β ratio might provide insights on the best operational strategy for improving the VOC mineralization in bioreactors devoted to air treatment. More studies applying the α/β ratio for K - and r -strategists are necessary to confirm the potential of the mathematical modelling approach here proposed.

5. Conclusions

A simple, accurate and easy-to-implement methodology for the kinetic characterization of VOC-degrading microorganisms was here presented. The mathematical approach used for the kinetic characterization indicated that the Luedeking–Piret correlation was suitable for describing the CO₂ production in the toluene biodegradation by *R. erythropolis*. This microorganism exhibited convenient characteristics for the treatment of low toluene gas concentrations supporting a very low K_S value, while supporting a K_I value comparable with those reported for *r*-strategist microorganisms. These particular characteristics can reduce the start-up time and increase the robustness of bioreactors devoted to air treatment. The methodology here developed can also be applied to other VOCs and microorganisms relevant for air pollution control bioreactors. The α/β ratio was a useful tool accounting for the relative relevance of biomass growth rate and biomass density on the VOC mineralization performance, providing insights towards the selection of the best operational strategy.

Acknowledgements

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