Biodegradation (2012) 23:81–92
DOI 10.1007/s10532-011-9488-7

ORIGINAL PAPER

Kinetics of biodegradation of diethylketone by *Arthrobacter viscosus*

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Received: 2 February 2011 / Accepted: 2 June 2011 / Published online: 17 June 2011

Abstract The performance of an *Arthrobacter viscosus* culture to remove diethylketone from aqueous solutions was evaluated. The effect of initial concentration of diethylketone on the growth of the bacteria was evaluated for the range of concentration between 0 and 4.8 g/l, aiming to evaluate a possible toxicological effect. The maximum specific growth rate achieved is 0.221 h^{-1} at 1.6 g/l of initial diethylketone concentration, suggesting that for higher concentrations an inhibitory effect on the growth occurs. The removal percentages obtained were approximately 88%, for all the initial concentrations tested. The kinetic parameters were estimated using four growth kinetic models for biodegradation of organic compounds available in the literature. The experimental data found is well fitted by the Haldane model ($R^2 = 1$) as compared to Monod model ($R^2 = 0.99$), Powell ($R^2 = 0.82$) and Loung model ($R^2 = 0.95$). The biodegradation of diethylketone using concentrated biomass was studied for an initial diethylketone concentration ranging from 0.8–3.9 g/l in a batch with recirculation mode of operation. The biodegradation rate found followed the pseudo-second order kinetics and the resulting kinetic parameters are reported. The removal percentages obtained were approximately 100%, for all the initial concentrations tested, suggesting that the increment on the biomass concentration allows better results in terms of removal of diethylketone. This study showed that these bacteria are very effective for the removal of diethylketone from aqueous solutions.

Keywords *Arthrobacter viscosus* · Biodegradation kinetics · Degradation · Diethylketone · Growth kinetics

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Introduction

Contamination of water streams with hazardous compounds has attracted increasing attention in recent decades all over the world. Many of these compounds, including aromatic hydrocarbons, aldehydes and ketones, are toxic to humans because of their carcinogenic and mutagenic properties and their capacity to form toxic and phytotoxic radical intermediates (Tani and Hewitt 2009).

The ketones are commonly employed in the manufacturing of paints, rubbers and pharmaceuticals (Raghuvanshi and Babu 2010a) and are also released by the gasoline, solvent and petrochemical industries (Raghuvanshi and Babu 2010b). Diethylketone is used as a solvent and as an intermediate in the synthesis of pharmaceuticals, flavors and pesticides.

The traditional technologies used for the treatment of chlorinated and ketones solvents are adsorption on

granular activated carbon (GAC), air-stripping and biofilters but GAC is prohibitively expensive to be used on industrial scale and air-stripping and biofilters are too expensive from operational and equipment perspectives.

Biological treatment of wastewater was often considered the most economical option when compared with other treatment options (Marco et al. 1997) and recently some efforts have been made through the use of biosorbents for the treatment of wastewaters contaminated with solvents (Osuna et al. 2008; Wu et al. 2007; Tsai et al. 2009; Wang and Tseng 2009). The possibility of wastewater to undergo biodegradation is dependent on many factors, such as concentration of contaminants and their chemical structure, water matrix, pH, and substrate/co-substrate ratio (Al-Momani et al. 2010).

Bacteria are well known for their use in the treatment of hazardous contaminated solutions (Quintelas et al. 2008). The structural polymers in the bacteria cell wall provide functional groups like carboxyl, phosphoryl and amino groups that are directly responsible for the reactivity of bacterial cells (Kulczycki et al. 2002). Xiao et al. (2007) affirm that as the interior plasma membrane is impermeable to organic pollutants, the bacterial cell wall is expected to be the primary element responsible for organic biodegradation. *Arthrobacter* sp. were used before on the removal of organic compounds with very promising results. Wu et al. (2010) used this bacterium to degrade di-n-octyl phthalate, Aresta et al. (2010) used *Arthrobacter sulfureus* isolated from olive-mill wastewater polluted soil to degrade phenol and Quintelas et al. (2006, 2010) used *Arthrobacter viscosus* supported on GAC to remove phenol, *o*-cresol and chlorophenol from contaminated wastewater.

This work aims the development of an environmental technology applicable to the treatment of aqueous solutions contaminated with solvents at low concentrations. The study is focused on the detailed kinetic study for the biodegradation of diethylketone. The effect of initial concentration of diethylketone (0 mg/l–4.8 g/l) on the growth of the bacteria was studied, aiming to evaluate a possible toxicological effect. The biomass was then concentrated ($\approx$7 g/l) and used for biodegradation studies. The diethylketone concentrations used for these biodegradation studies were on the range 0.8–3.9 g/l. The information collected from these experimental studies was then used for the calculation of growth kinetic constants and of rate kinetic constants from different models—Monod (Monod 1949), Powell (Powell 1967), Haldane (Andrews 1968), Luong (Luong 1986) kinetic models—reported on the literature as models with applications for the biodegradation of organic pollutants. The significance of this work is notorious because it defends a quite straightforward technology to remove diethylketone from contaminated aqueous solutions using microorganisms. This process is sustainable, of easy maintenance and very competitive with the most traditional procedures to remove solvents from wastewater. The removal of organic solvents from contaminated systems is a problem of particular concern. In fact, these substances are subject to increasing severe environmental constraints due to their direct danger to and impact on health and environment.

Materials and methods

Materials

Aqueous diethylketone (3-pentanone) solutions were prepared by diluting diethylketone (Acros Organics) in distilled water. The microorganism used was *Arthrobacter viscosus* (CECT 908) from the Spanish Type Culture Collection—University of Valencia. All the media nutrients were of analytical grade—peptone and glucose (Riedel), yeast extract (Fluka), malt extract (Biokar Diagnostics).

Methods

Media preparation

The media prepared have the following composition: peptone—5 g/l, malt extract and yeast extract—3 g/l, glucose—10 g/l, in distilled water. The pH was adjusted to 7.

Effect of initial concentration of diethylketone on the growth: toxicological assays

A reactor (750 ml) was filled with 500 ml of this medium and autoclaved. The reactor was then inoculated with a pure culture of *Arthrobacter*

viscosus and an amount of diethylketone was added (1.6–4.8 g/l). A control experiment without diethylketone was also performed. The reactor was equipped with a heating jacket to keep the temperature constant (28°C). The agitation was implemented by medium recirculation and by a magnetic stirrer. At different time intervals the optical density (OD) was measured at 620 nm (T60 UV-Visible Spectrophotometer, PG Instruments). The initial concentration of diethylketone was quantified using GC (Chrompack CP 9001) equipped with a flame ionization detector (FID). All the assays were done in duplicate and the results presented are an average of both assays. The relative standard deviation and relative error of the experimental measurements were less than 2 and 5%, respectively.

Biodegradation study

Arthrobacter viscosus was grown for 24 h at 28°C in a culture medium with the composition described in 2.2.1 (500 ml) and then 150 ml (×3) of this inoculated medium were transferred to 3 new culture media (1000 ml) and these cultures were grown for 48 h. After this period, the biomass was centrifuged on a Sigma 4K15 centrifuge (RCF of 7950) and the supernatant was collected on a sterile bottle for later use. The biomass pellets were re-suspended on a smaller volume of the collected medium, being this volume calculated to obtain a final biomass concentration in culture up to 7.0 $g_{biomass}$/l. The reactors filled with a diethylketone solution (500 ml) were then inoculated with this previous concentrated biomass. The diethylketone concentrations used for these studies were on the range 0.8–3.9 g/l. At different time intervals, a sample was taken, centrifuged at 13400 rpm for 10 min, and the supernatant was used for the estimation of diethylketone concentration. The assays were conducted in duplicate, during 4–6 days. The results presented are an average of both assays. The relative standard deviation and relative error of the experimental measurements were less than 2 and 5%, respectively.

Characterization procedures

The GC employed herein was a Chrompack CP 9001, equipped with a flame ionization detector (FID), and separations were performed using a TRB—Wax capillary column (30 m×0.32 mm i.d × 0.25 μm). The operating conditions were as follows: the column was held initially at a temperature 50°C, then heated at 10°C/min to 100°C, held at 100°C for 4 min, then heated again at 40°C/min to 200°C and finally held at 200°C for 2 min. The temperature of injector and detector were maintained at 250°C. Nitrogen was used as a carrier gas at a flow rate of 30 ml/min and the injections were performed in the split mode with a split ratio of 1:14. Under these conditions, the retention time for ketone was 2.2 min.

Modelling the growth kinetics

In the present study, the linear and nonlinear growth kinetic models were fitted by methods of linear and nonlinear least squares using MATLAB software. The models used were Monod, Powell, Haldane and Loung kinetic models.

Monod model

Along the last decades, the microbial growth kinetics has been modelled by the well known Monod model (Monod 1949). With this model, Monod introduced the concept of a growth-controlling or limiting substrate where the specific growth rate (μ) is related to the concentration of a single growth-controlling substrate (S) via two parameters, the maximum specific growth rate (μ_m) and the substrate affinity constant (Ks) as given by Eq. 1.

$$\mu = \frac{\mu_m * S}{Ks + S} \quad (1)$$

Powell model

Powell (1967) modified the Monod equation by introducing the term of maintenance rate (m), as expressed on Eq. 2.

$$\mu = \frac{(\mu_m + m) * S}{Ks + S} - \text{ m} \quad (2)$$

Monod and Powell models do not consider the self inhibition effect which was exhibited during the diethylketone biodegradation process. Various substrate inhibition models such as Haldane (Andrews 1968) and Luong models (Luong 1986) were also used in the present study.

Haldane model

The Haldane equation, due to its mathematical simplicity and wide acceptance for representing the growth kinetics in response to inhibitory substrates, was appropriate for describing *Arthrobacter viscosus* growth kinetics in diethylketone. The specific growth rate (μ) for batch growth can be modelled as:

$$\mu = \frac{\mu_m * S}{Ks + S + (S^2/K_I)} \tag{3}$$

K_I represents the self-inhibition constant. A high K_I value implies that the culture is not sensitive to substrate inhibition (Li et al. 2010).

Luong model

The inhibitory effect of the substrate on biomass growth under batch conditions can also be expressed by the Luong model. This model is based on certain assumptions which include no lag phase, organism death, endogenous respiration, substrate used for maintenance energy and inhibition by products (Raghuvanshi and Babu 2010a). The model incorporates a term, given as S_m critical inhibitor concentration, above which the growth is completely inhibited (Eq. 4).

$$\mu = \frac{\mu_m * S}{Ks + S}\left(1 - \frac{S}{S_m}\right)^n \tag{4}$$

S_m is the critical inhibitor concentration above which biodegradation stops and n is the Loung coefficient.

Modelling of the biodegradation kinetics

The experimental removal data were modelled using zero order, pseudo-first, pseudo-second and three-half order equations. The linearized form of the zero order model (Shoaib et al. 2006), pseudo-first order model (Khamis et al. 2009), pseudo-second order model (Al-Ghouti et al. 2009) and three-half order model (Saravanan et al. 2009) are shown below as Eqs. 5–8, respectively:

$$\text{Log } S = \text{Log } S_0 - \frac{K_0 * t}{2,303} \tag{5}$$

$$\log(q_e - q_t) = \log(q_e) - k_1 * t \tag{6}$$

$$\frac{t}{q_t} = \frac{1}{K_2 * q_e^2} + \frac{t}{q_e} \tag{7}$$

$$Y = -K_{31} - \frac{K_{32}}{2} * t \tag{8}$$

where

$$Y = \frac{1}{t} * \frac{\ln(S_0 - P + K_0 * t)}{S_0} \tag{9}$$

and

$$P = S_0 - S + K_0 * t \tag{10}$$

S_0 and K_0 are constants calculated by zero order kinetic model, q_e is the amount of diethylketone removed at equilibrium (mg/g), q_t the amount of diethylketone removed at time t (mg/g), K_1 is the pseudo-first order rate constant, K_2 is the pseudo-second order rate constant and K_{31}, K_{32} are the three-half order rate constants.

Results and discussion

In the present study, the growth of *Arthbacter viscosus* on the presence of diethylketone was studied for the initial concentrations of 0–4.8 g/l in order to evaluate a possible xenobiotic effect. The biomass was then concentrated and used for biodegradation studies. The effect of the concentration of the biomass and the effect of the diethylketone initial concentration on biodegradation is discussed below.

Effect of initial concentration of diethylketone on the growth

Figure 1 shows the biomass concentration profile at different times for initial diethylketone concentration ranging from 0–4.8 g/l. Biomass concentration was calculated using a calibration curve (optical density vs. biomass concentration). Biomass concentration showed a typical growth curve (Prescott et al. 2002) that can be categorized in phases: lag, log, stationary and death phase as shown in Fig. 2, for the growth with no diethylketone. During the lag phase, cells undergo intracellular changes in an effort to adjust to a new environment and little or no cell reproduction takes place. During the exponential phase, cells reproduce at a rate proportional to the number of cells leading to an exponential increase in the number of cells. The stationary phase follows when nutrients are limited or other environmental conditions restrict

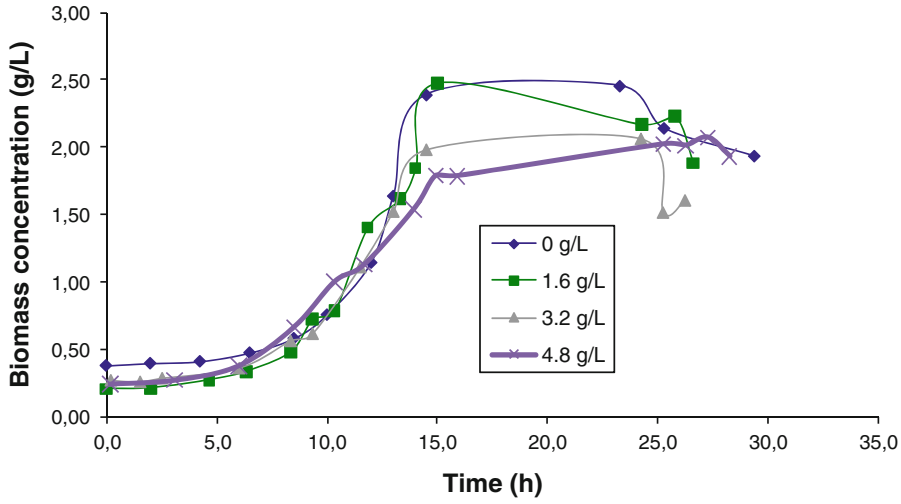

Fig. 1 Biomass concentration (g/l) versus time (h) for different initial diethylketone concentrations

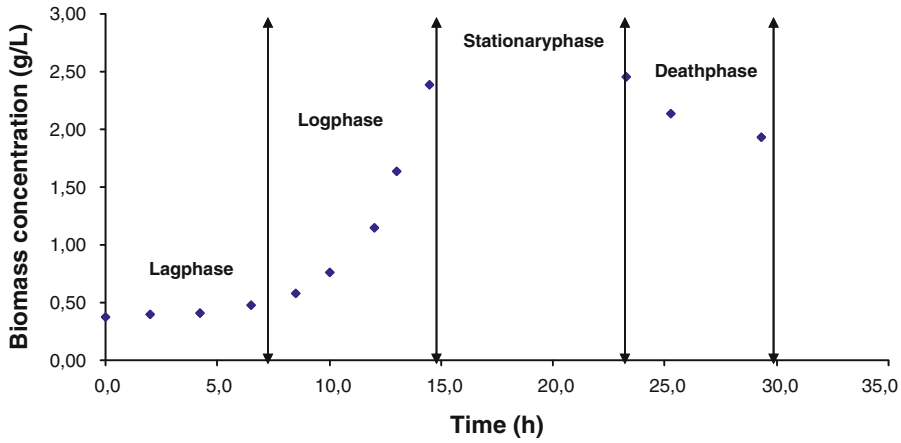

Fig. 2 Different growth phases from the growth curve of *Arthrobacter viscosus* performed without diethyketone

the number of cells that can be supported. Finally, cellular death and a declining population occur when the surroundings cannot maintain the population (Yates and Smotzer 2007).

During lag phase, no difference was found with the increase of the initial concentration of diethylketone. All the concentrations tested present the same profile which means that the bacteria take the same time for acclimation to the new environment. In log phase, the biomass concentration increased rapidly as can be seen in Fig. 1, for all the concentrations tested. However, for the concentrations of 3.2 and 4.8 g/l, the log phase is shorter (in terms of biomass concentration) which means that the bacteria grow less and the most logical reason is that despite the fact that diethylketone is a carbon source and theoretically it allowed better conditions for bacterial growth, the strong toxic properties of the diethylketone promote a xenobiotic/inhibitory effect on the bacteria.

The maximum biomass concentrations were obtained as 2.46, 2.48, 2.06 and 2.07 g/l for initial diethylketone concentrations of 0, 1.6, 3.2 and 4.8 g/l, respectively.

Despite the fact that, at this point, the aim of this work was to study the toxicological effect of diethylketone on the growth of the bacteria, the consumption of diethylketone was also quantified. The diethylketone concentration was found to decrease with time showing the consumption of diethylketone by the bacteria. The removal percentages obtained were approximately 88%, for all the assays.

It is important to highlight that diethylketone is not the only carbon source present on these assays. The

main carbon source is the glucose present on the culture media. On the next set of assays, performed to analyse the biodegradation of diethylketone, the biomass was first concentrated to an amount of 7–8 g/l and this concentrated biomass was added to a diethylketone solution where the only carbon source is the diethylketone.

Biodegradation assays

The biomass concentration for the second set of assays was calculated using a calibration curve (optical density vs. biomass concentration) and the measurement of the dry weight. It was decided to use concentrations of diethylketone lower than 4.8 g/l, for the biodegradation studies. The concentrations of diethylketone used were 0.8, 1.6, 2.4, 3.2 and 3.9 g/l. Despite the fact that the previous assays (point 3.1) showed that a concentration of 3.2 g/l presents already a xenobiotic effect, the higher concentration of the biomass (smaller ratio solvent/biomass) could avoid this effect and to show that it was decided to use solvent concentrations of 3.2 and 3.9 g/l. The concentrated biomass was added to a diethylketone solution (only diethylketone diluted on water) to avoid any interference of other carbon sources.

Figure 3 shows the diethylketone removal percentage vs time, for all the diethyketone concentrations tested and Fig. 4 shows the time profile of diethylketone, for the initial concentration of 1.6 g/l. The removal is fast on the first 20 h, after that it slows down until 100% are reached (for concentrations higher than 0.8 g/l). This initial input is due to the availability of the biomass and to the need of the biomass to consume a carbon source. It is important to highlight that this initial input decreases as the initial concentration of diethyketone increases (Fig. 3). This decrease is justified by the saturation of the biomass, or in other words, it is due to the concentration increase while the biomass concentration is approximately the same for all the assays, meaning less availability of the biomass to degrade diethylketone.

The degradation of the diethylketone is well demonstrated on Fig. 5, as an example fot the initial concentration of 2.4 g/l. In fact, the biomass grows slightly with the consumption of diethylketone and decreases as the diethylketone disappears. The death or inactivity of the biomass occurs for all the initial concentrations higher than 1.6 g/l and could also be related to the toxicological effect of the diethylketone. For the concentration of 0.8 g/l the decrease on the biomass concentration was not detectable.

The metabolic activity during the biodegradation of two or more pollutants may involve induction, inhibition or co-metabolism, depending on the substrates and microbial species present (Chan and Lai 2010). Usually, enzymes are responsible for the degradation of hazardous organic compounds. *Arthrobacter* species are well known to produce enzymes as NADH oxidases, catalases, chitanases (Hofsten and Reinhammar 1965; Lonhienne et al. 2001; Meganathan and Ensign 1976) that could be used for degradation of

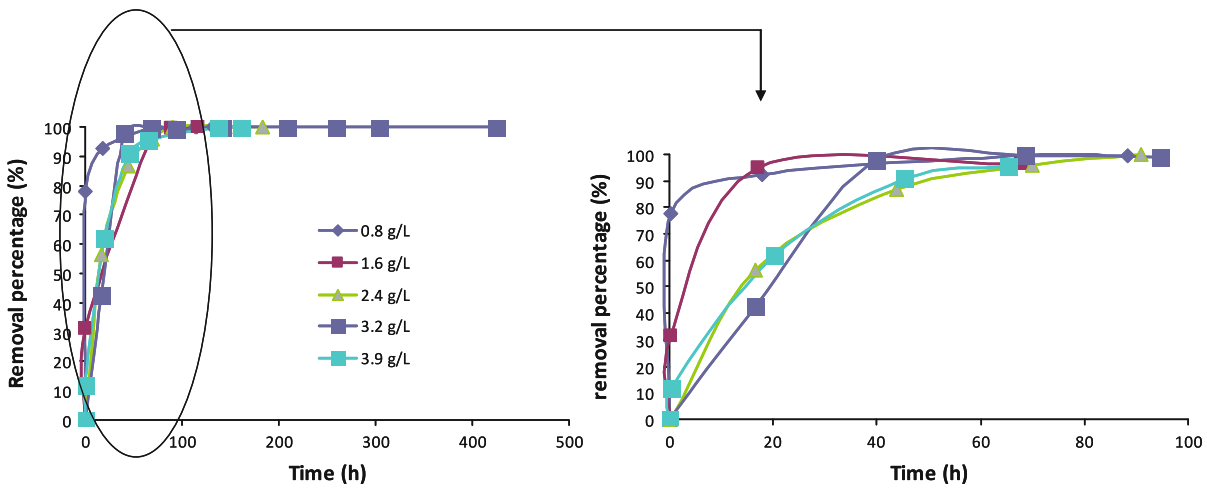

Fig. 3 Removal percentage versus time, for all the diethyketone concentrations tested

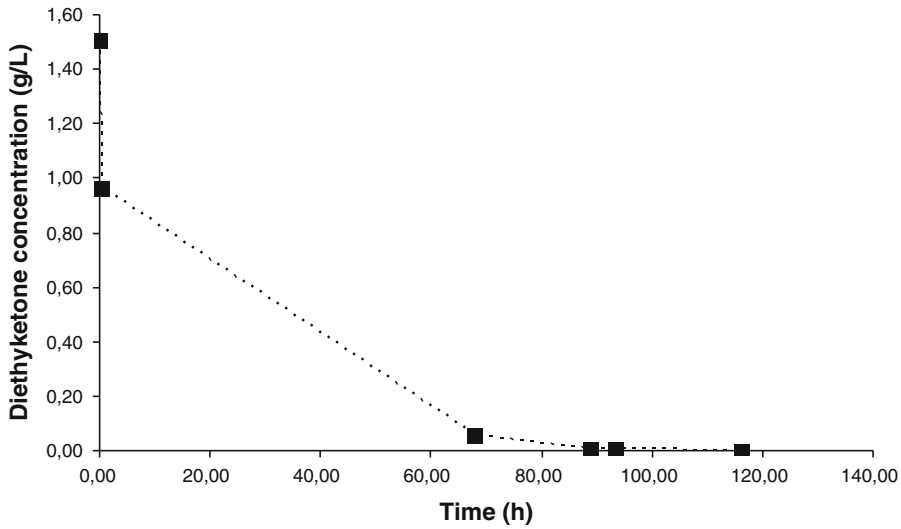

Fig. 4 Time profile of diethylketone for an initial concentration of 1.6 g/l

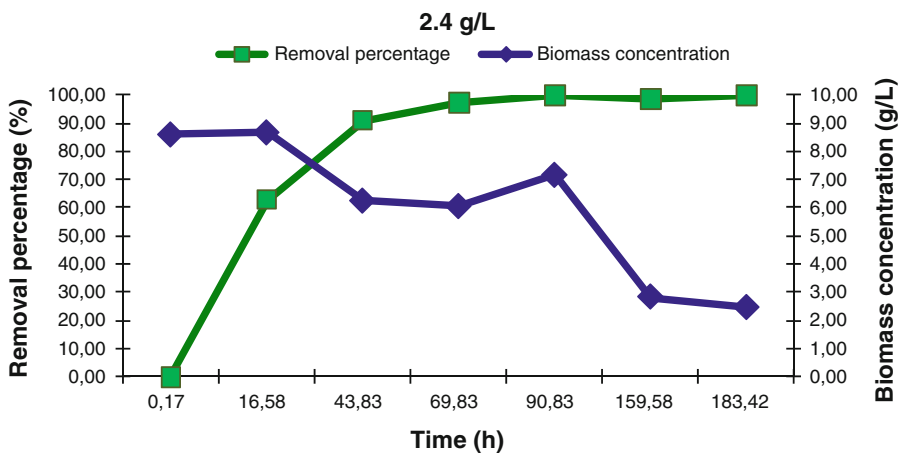

Fig. 5 Biomass concentration (g/l) and removal percentage (%) as functions of time, for the diethylketone concentration of 2.4 g/l

pollutants. In parallel to the enzymatic induction, the binding of the hazardous compounds to functional groups present on the cell walls of the bacteria may also contribute to the removal of these compounds. Accordingly to previous studies (Lameiras et al. 2008), the main functional groups of *Arthrobacter viscosus* biomass include bonded hydroxyl group and –NH, C–N and N–H, COO^- anions, $–SO_3$ and –CH groups. These groups could interact with diethylketone by physical binding, such as electrostatic and van der Waals forces or chemical binding, such as ionic and covalent binding (Won et al. 2008).

During the GC analysis an intermediate appears after some hours of experiment. This intermediate was later identified as 2-methyl-1-propanol proving that the diethylketone is being biodegraded. Similar events were also observed by other authors (Przybulewska and Wieczorek 2009). This intermediate appeared on both, toxicological assays and biodegradation assays, but it was also biodegraded by the concentrated biomass. On the toxicological assays the intermediate remains at the end of the assay because the biomass had not time to degrade it (the toxicological assays were performed during 1–1.5 days and the biodegradation assays 4–6 days).

The removal percentages obtained with the concentrated biomass were 100%, for all the assays and the uptake values are 96.5, 235, 339, 410 and 491 $mg/g_{bacteria}$ respectively for the initial concentrations of 0.8, 1.6, 2.4, 3.2 and 3.9 g/l. The increment on the biomass concentration allowed better results in terms of removal of diethylketone.

Modelling the growth kinetics of *Arthrobacter viscosus* in the presence of diethylketone

The degradation of diethylketone leads to the formation of biomass and as diethylketone degradation is the result of the microbial activity, the kinetics of diethylketone degradation is closely related to the kinetics of microbial growth. The obtained biomass concentrations at different time intervals for various initial concentrations of diethylketone were used to calculate the specific growth rate accordingly to Eq. 11.

$$\mu = \frac{1}{C}\frac{dC}{dt} \tag{11}$$

where, μ is the specific growth rate (h^{-1}), C is the biomass concentration (g/l) at time t (h), and dt is differential change in time (h) for the differential change in biomass concentration, dC. After integration, Eq. 11 can be represented by Eq. 12:

$$\ln C = \ln C_0 + \mu t \tag{12}$$

C_0 is the initial biomass concentration (g/l) at t = 0. A linear least square method was used to calculate the specific growth rate using the data obtained for log phase at different initial concentrations of diethylketone. A plot of ln C versus t gives a straight line with ln C_0 as its intercept and μ as the slope (Fig. 6). The obtained values of specific growth rates are 0.2065, 0.2209, 0.1737 and 0.1581 h^{-1}, respectively for the initial diethylketone concentrations of 0, 1.6, 3.2 and 4.8 g/l. The decrease verified on the specific growth rate for initial diethylketone concentrations higher than 1.6 g/l suggested inhibition by diethylketone.

Carbon substrates as ketones are most often utilized by microorganisms simultaneously under the carbon and energy controlled environmental conditions. Since growth is a result of catabolic and anabolic enzymatic activities, these processes, i.e., substrate utilization or growth-associated product formation, can also be quantitatively described on the basis of growth models (Raghuvanshi and Babu 2010a). The specific growth rate (μ) of a population of microorganisms and the substrate concentration (S) could be related by a set of empirically derived rate laws which are considered as theoretical models. Various theoretical models such as Monod, Powell, Haldane and Luong kinetic models were used on this study and the growth kinetic parameters obtained for the different growth models are presented in Table 1.

For the Monod model the values of μ_m and Ks were calculated from the plot of $1/\mu$ vs. 1/S, which the intercept of the line gave the value of μ_m and the slope gave Ks. The values of μ_m and Ks were obtained as 0.1398 h^{-1} and 0.591 g/l respectively (Table 1). The obtained value of correlation coefficient (R^2) was 0.99 which indicates that this model fits properly the data. The predicted values of specific growth rate at different substrate concentration values are given in Table 2.

The inhibition of diethylketone destruction was observed above the 1.6 g/l of initial diethylketone concentration as the specific growth rate decreased above this concentration (Fig. 1; Table 2 (μ_{exp})). The

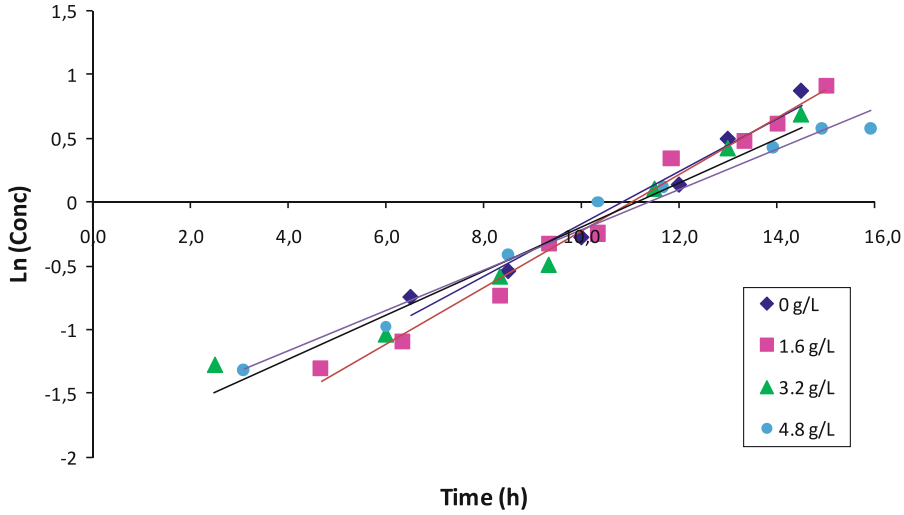

Fig. 6 Ln (Concentration) as a function of time for the determination of the specific growth rate

Table 1 Growth kinetic parameters obtained for the growth kinetic models used

Model	μ_m (h^{-1})	Ks (g/l)	K_I (g/l)	S_m (g/l)	m	n	R^2
Monod	0.1398	0.591	–	–	–	–	0.99
Powell	0.1444	0.024	–	–	4,978	–	0.82
Haldane	0.1593	0.506	42.36	–	–	–	1.00
Luong	0.1095	0.739	–	9.65E6	–	5.1E6	0.95

Table 2 Experimental and predicted values of specific growth rate for the growth kinetic models used

S_0 (g/l)	μ_{exp} (h^{-1})	$\mu_{predicted}$ (h^{-1})			
		Monod	Powell	Haldane	Loung
1.6	0.2209	0.2217	0.2172	0.2208	0.2220
3.2	0.1737	0.1715	0.1811	0.1736	0.1554
4.8	0.1581	0.1594	0.1689	0.1581	0.1412

value of Ks was much smaller than the lowest concentration used in this study which also indicated the inhibition of diethylketone (Raghuvanshi and Babu 2010a).

For Powell model, the three kinetic constants obtained were 0.1444 h^{-1}, 0.024 g/l and 4.978, respectively for μ_m, Ks and m (Table 1). The obtained value of correlation coefficient (R^2) was 0.82 which indicates that this model does not fit properly the data and fits even worse than the Monod model despite the fact of the incorporation of maintenance rate in Powell model. Table 2 shows the comparison between experimental data and Powell model predicted data.

As it was explained on "Materials and Methods" section, Monod and Powell models do not consider the inhibition effect which was exhibited during the diethylketone biodegradation process. As it was said before, if the substrate concentration is much higher than the affinity constant values (Ks), substrate inhibition models do describe better the kinetic growth. For Powell model the value of Ks obtained is also much less than the smaller diethylketone concentration, as for the Monod model which confirms the inhibition.

The constants for Haldane model (Eq. 3) were calculated and are listed on Table 1 and the fit of Haldane model to the experimental results and the predicted values of specific growth rate at different concentration are listed in Table 2. The value of correlation coefficient ($R^2 = 1$) showed that the present data is completely fitted by the Haldane model. On Table 2 is possible to see that the μ_{exp} is equal to the $\mu_{predicted}$.

For Loung model, the kinetic constants were obtained and listed on Table 1. The obtained value of correlation coefficient ($R^2 = 0.95$) which indicates a relatively good agreement with the experimental data. Table 2 shows the experimental results and the predicted values of specific growth rate at different concentrations, for the Loung model.

The discussion of the results proves that the inhibition model of Haldane explains better the growth kinetics in the presence of diethylketone than the others models used.

Modelling the removal kinetics of diethylketone by *Arthrobacter viscosus*

The removal kinetics is very important for the process design and operation control of a removal process. In biodegradation processes, several kinetic approaches describing the transformation of organic compounds by suspended microorganisms have being evaluated. Zero order, pseudo-first order, pseudo-second order and three-half order rate kinetics are some of them. The constants for all models were evaluated and listed in Table 3 and the corresponding plots are shown in Fig. 7.

The pseudo-first order model assumes that the reaction rate is limited by the process on a single

Table 3 Constant parameters of zero-order, pseudo-first, pseudo-second and three-half-order kinetic models for different initial diethylketone concentrations

S (g/l)	Zero order kinetics			Pseudo-first order kinetics	
	K_0 (g/(l h))	S_0 (g/l)	R^2	K_1 (h)	R^2
0.8	0.0461	0.52	0.971	1.1298	0.996
1.6	0.0585	1.35	0.965	0.6883	0.962
2.4	0.0546	1.47	0.936	0.4876	0.943
3.2	0.0520	0.79	0.778	0.4148	0.995
3.9	0.0424	2.53	0.990	0.7143	0.830

S (g/l)	Pseudo-second order kinetics		Three-half order kinetics		
	K_2 (g/(mg h))	R^2	K_{31} (1/h)	K_{32} (1/h^2)	R^2
0.8	3.7668	0.998	0.0697	−0.0006	0.999
1.6	4.8701	0.999	0.0327	−0.0002	0.999
2.4	0.9914	0.999	0.0336	−0.0004	0.837
3.2	0.3990	0.996	0.0669	−0.0006	0.799
3.9	0.4928	0.999	0.0181	−0.0002	0.801

class of sorbing sites and that all sites are time dependent (Fonseca et al. 2009). The correlation coefficients varied between 0.83 and 0.996. The constant K_1 was found to decrease except for the initial concentration 3.9 g/l.

In the case of the pseudo-second order model, the correlation coefficients were found to be around 0.995–0.999, which indicate a good fit of the experimental data. The constant K_2 increased for the initial concentration 1.6 g/l and then decreased with the increase on the diethlketone concentration.

The last two models, pseudo-first and pseudo-second order, have the limitation that they do not take into account the bacterial growth. Brunner and Focht (1984) proposed a model named three-half-order kinetic model trying to solve this limitation. The model was based on the first-order model with the introduction of an additional term to explain the biomass formation (Raghuvanshi and Babu 2010a). The P on Eq. 10 is the rate of product formation (CO_2) which is directly related to the change in biomass concentration. K_0 and S_0 are zero order rate constants and can be obtained by the zero-order kinetic model, Eq. 5. The zero order and three-half order kinetic constants were evaluated and listed in Table 3 and the corresponding plots are shown in Fig. 6. The correlation coefficient obtained for zero-order kinetics was found in the range of 0.836–0.99 for various initial diethylketone concentration values. The value of S_0 was found to increase with the increment on the diethylketone concentration, except for the concentration 3.2 g/l and K_0 increased till the concentration 1.6 g/l and then decreased.

The obtained value of three-half-order rate constants (K_{31} and K_{32}) were calculated and the obtained value of regression correlation coefficient ($R^2 =$ 0.799–0.999) indicated that the three-half-order kinetic model, despite the introduction of the additional term for explaining the biomass formation, did not allow better results than the pseudo-second order model.

Conclusions

The study is focused on the growth kinetics of an *Arthrobacter viscosus* culture and on the kinetics of simultaneous degradation of diethylketone. The effect of initial diethylketone concentration on the growth curves shows that concentrations higher than 1.6 g/l inhibited the bacteria growth. The different growth kinetic models such as Monod, Powell, Haldane and Luong were tested. The obtained value of the correlation coefficient for Haldane model suggested that this model suits to the biodegradation kinetics of diethylketone better than the other models.

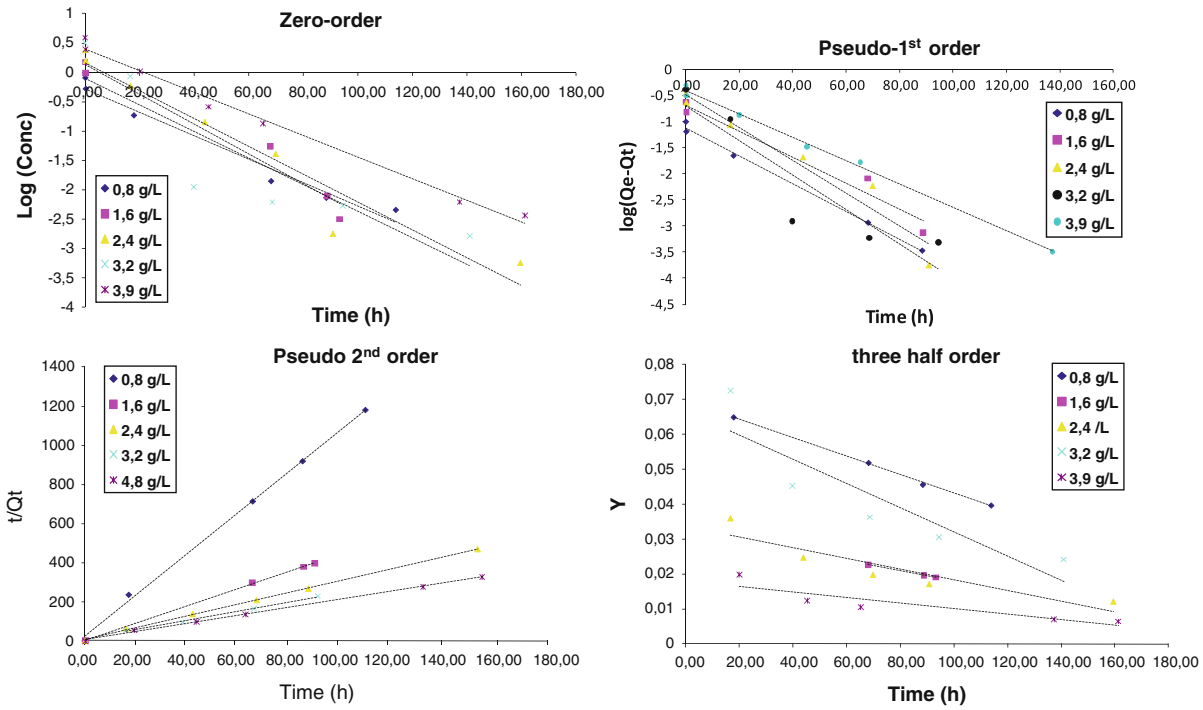

Fig. 7 Plot of zero order, pseudo-first order, pseudo-second order and three-half order kinetics for the removal of diethylketone

The removal percentages obtained were approximately 88%, for all the initial concentrations tested. The biomass was then concentrated and tested for the removal of diethylketone and removal percentages of 100% were found for all the initial concentrations. Zero order, pseudo-first order, pseudo-second order and three-half order models were tested for the biodegradation assays and pseudo-second order kinetic model was found suitable for the biodegradation of diethylketone.

Acknowledgments The authors would like to gratefully acknowledge the financial support of this project by the Fundação para a Ciência e Tecnologia (FCT), Ministério da Ciência e Tecnologia, Portugal and Fundo Social Europeu (FSE). Cristina Quintelas thanks FCT for a Post-Doc grant.

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