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ORIGINAL ARTICLE

Relevance of the Luong model to describe the biodegradation of phenol by mixed culture in a batch reactor

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Abstract The kinetics of phenol biodegradation by mixed culture in a batch reactor was investigated over a wide range of initial phenol concentrations (40–350 mg L^{-1}). The temperature (30°C), the stirring velocity (200 rpm), mineral salt supplementation, namely NaH_2PO_4 (3 g L^{-1}), KH_2PO_4 (3 g L^{-1}), $MgSO_4$ (0.1 g L^{-1}), $(NH_4)_2SO_4$ (1 g L^{-1}), as well as the initial pH (8) were kept constant. All experiments were carried out at a given initial bacterial concentration of 0.08 g L^{-1} (based on the optical density determination, 0.079). Irrespective of the culture conditions, total phenol degradation was recorded for a culture time ranging from 15.6 to 49.0 h. In addition, the optimal value of the maximum specific growth rate was observed for 125 mg L^{-1} of phenol. These results show the relevance of the specific microbial consortium used. The Luong equation accurately matched growth kinetics and enabled the relation between the maximum specific growth rates (μ_m) and the initial phenol concentration (S_o) to be described. It could also be used to deduce the substrate saturation coefficient (K_s) and the maximum substrate concentration (S_m) above which growth ceases. The kinetic constants of the Luong equation for phenol were $\mu_m = 1.04\,h^{-1}$, $K_s = 153.2\,mg\,L^{-1}$, $S_m = 540\,mg\,L^{-1}$ and $n = 0.9$, respectively.

Keywords Biodegradation · Phenol · Microbial consortium · Growth kinetics

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Introduction

Effluents of industrial wastewaters from oil refineries, paper mills, dyes, ceramic factories, resins, textiles, and plastic contain high concentrations of aromatic compounds that are toxic to living organisms. Degradation of these compounds to achieve tolerant levels prior to their release into the environment is therefore an urgent requirement. There are many published reports describing a wide variety of ways to degrade these effluents. Of these, microbial biodegradation has proved to be an effective and economically viable method for the treatment of such contaminants. Researchers have isolated and identified a number of microbes for the oxidation of phenols (Rigo and Alegre 2004; San-Chin et al. 2005; Santos and Linardi 2004; Vijayagopal and Viruthagiri 2005a).

Numerous culture methods, involving various types of microorganisms, have been used to treat phenol, such as substrate inhibition of phenol oxidation (Bergauer et al. 2005; Claussen and Schmidt 1998; Goswami et al. 2005; Kennes and Lema 1994; Luke and Burton 2001; Millette et al 1998; Perron and Welander 2004; Stephenson 1990; Yu and Loh 2002), the protoplast fusion technique (Chang et al. 1995; 1998), and the degradation of phenol in the presence of other

derivatives (Komarkova et al. 2003). In all of the studies reported to date, phenol was metabolized by the growing microbes and used as carbon and energy source.

To describe substrate biodegradation, it is necessary to evaluate the relationship between the specific growth rate, μ, and the initial phenol concentration, S_0. Phenol biodegradation by bacteria is generally inhibited by phenol itself. The Haldane and Monod equations have frequently been used to describe this degradation in pure or mixed cultures (Allsop et al. 1993; Beyenal et al. 1997; D'Adamo et al. 1984; Hill and Robinson 1975; Lallai et al. 1988; Pawlowsky and Howell 1973; Sokol 1987; Sokol and Howell 1981; Szetela and Winnicki 1981; Tang and Fan 1987; Yang and Humphrey 1975;).

The model proposed by Luong (1987) and given in Eq. (1) appears to be useful to describe the kinetics of substrate inhibition. Indeed, the proposed model is of the generalized Monod type, and in addition to being able to describe substrate limitation observed at a low concentration (Monod model), it also allows substrate inhibition observed at a high concentration to be accounted for through the parameter S_m, the maximum substrate concentration, above which growth is completely inhibited (Luong 1987).

$$\mu = \frac{\mu_m S}{K_S + S}\left(1 - \left(\frac{S}{S_m}\right)\right)^n \quad (1)$$

In this equation, S is the substrate concentration, μ is the specific growth rate and μ_m is its maximum value, K_s is the substrate saturation coefficient, and S_m is the maximum substrate concentration above which growth ceases.

The main goal of this paper was to investigate the biodegradation of phenol by a microbial consortium. To this end, we used ammonium sulphate as a nitrogen source. The novelty of this work can be detailed in two aspects, namely, the effect of the time of the lag phase t_0 on the specific growth rate, and the determination of the Luong model coefficients, which was compared to the well-known Monod and Haldane models.

Materials and methods

Cultivation of microorganisms

The mixed bacteria used in this work were obtained from activated sludge from the hazardous wastewater station of Boumerdès (Algeria). The stock cultures were stored at 4° C. The mixed bacteria were activated for 24 h at 30°C in nutrient medium [NB; (g L^{-1}) peptone, 15; yeast extract, 3; sodium chloride, 6; and (D+)-glucose, 1].

After 24 h, when cells had grown, the biomass was harvested by centrifugation at 3000 rpm for 30 min, and the microorganisms were suspended in 0.5% NaCl and re-centrifuged. The washing/harvesting procedure was carried out three times. Following the last washing, the microorganisms collected after centrifugation were re-suspended in 0.5% NaCl to determine the concentration of the mixed bacteria. This solution (mixed bacteria and 0.5% NaCl) was analyzed by measuring optical density (OD) at 600 nm using a Vis spectrophotometer (model DR2800; Hach, Loveland, CO); the OD value was then converted to dry cell mass using a dry weight calibration curve. The dry cell mass density (g L^{-1}) was found to follow the following regression equation × (g L^{-1})=1.044×OD_{600}.

Biodegradation experiments

When the OD value of the cells reached 2.7–2.9, an aliquot of the culture was centrifuged at 3000 rpm for 30 min. The biomass was re-suspended in 0.5% NaCl for washing and then centrifuged. The harvested cells (1 mL) were transferred and inoculated into Erlenmeyer flasks (250 mL) containing 100 mL of medium comprising a nitrogen source [$(NH_4)_2SO_4$, 1 g L^{-1}], mineral salt supplementation (MSS) in the form of NaH_2PO_4 (3 g L^{-1}), KH_2PO_4 (3 g L^{-1}), and $MgSO_4$ (0.1 g L^{-1}), and 40–350 mg L^{-1} of phenol, to an initial OD of 0.078. The cells were cultivated at 30°C and 200 rpm. Samples (1.4 mL) were withdrawn at suitable time intervals, and the concentration of cells was deduced from OD measurements at 600 nm. The phenol concentration was measured as described below.

Residual phenol determination

Phenol was colorimetrically estimated using a Vis spectrophotometer (model DR2800; Hach) according to the method previously described by Yang and Humphrey (1975) and based on rapid condensation with 4-aminoantipyrine followed by oxidation with alkaline potassium ferricyanide and absorbance reading at 510 nm.

Results and discussion

For each initial phenol concentration, S_0, the specific growth rate, μ, was determined in the exponential growth phase (Dagley and Gibson 1965; Worden and Donaldson 1987). Small inoculum volumes were used, so that the substrate concentration could be assumed constant in this initial growing period. For each flask, μ was determined from the slope of linear semi logarithmic plots of OD versus time during the exponential growth phase, where μ was approximately constant (D'Adamo et al. 1984).

Figure 1 shows a range of optical microbial growth curves where time-courses of OD are plotted for various

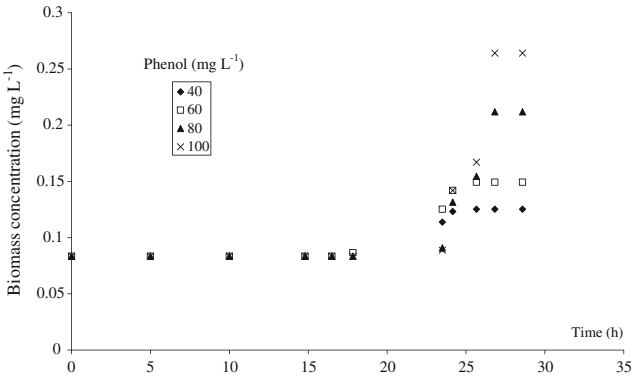

Fig. 1 Time-courses of biomass concentration at various initial phenol concentrations

initial phenol concentrations. As expected, a higher phenol concentration yielded a higher biomass concentration at the end of the exponential growth phase.

For initial phenol concentrations in the range 40–350 mg L^{-1}, maximum specific growth rates displayed a maximum value of 0.64 h^{-1} for S_0=125 mg L^{-1}.

The initial pH value was equal to 8. A slight decrease was observed with increasing biomass, but it was always less 10% of the initial pH value. The pH increased after the exponential growth phase, in agreement with results reported by Lallai et al. (1988).

The influence of the phenol concentration on the duration of the lag phase is shown in Fig. 1. The time of the lag phase, t_0, corresponds to the time for which the biomass concentration remains at its initial value.

The time of the lag phase t_0 increased exponentially with the phenol concentration (Fig. 2) in the range of concentrations tested, namely, 40–350 mg L^{-1}, leading to the conclusion that high concentrations of phenol have an inhibitory effect on microbial growth. These results are in agreement with those reported previously for mixed cultures (Lallai et al. 1988; Pawlowsky and Howell 1973). This exponential trend is also in agreement with the findings of Dapaals and Hill (1992). Using a pure culture of *Pseudomonas putida*, Alvaro et al. (2000) observed that the time of the lag phase increased linearly for phenol concentration in the range 60–600 mg L^{-1}.

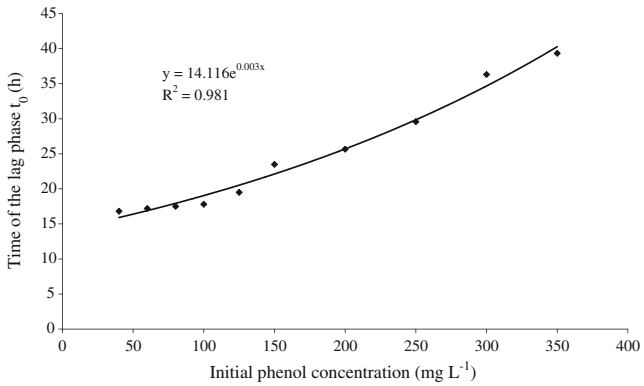

Fig. 2 Time of the lag phase (t_0) versus the initial phenol concentration (S_0)

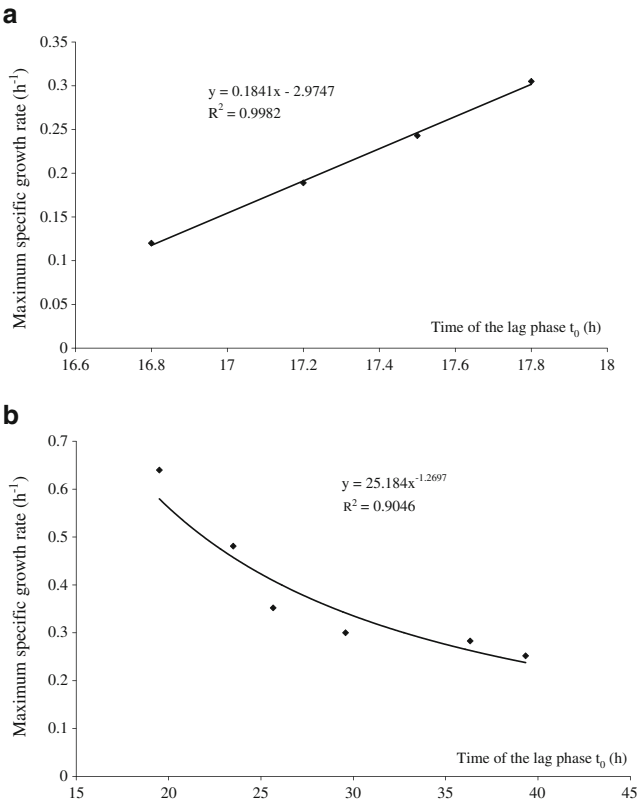

Fig. 3 Maximum specific growth rate μ_m versus the time of the lag phase t_0 for S_0 below (**a**) and above (**b**) a phenol concentration of 125 mg L^{-1}

To learn more about the effect of the lag phase, we examined its evolution versus the specific growth rate. Two trends were observed—one below (Fig. 3a) and one above (Fig. 3b)

Table 1 Maximum specific growth rate and time of the lag phase for various initial phenol concentrations

Initial phenol concentration S_0 (mg L^{-1})	Maximum specific growth rate μ_m (h^{-1})	Time of the lag phase t_0 (h)	Model
40	0.12	16.8	$\mu_m = 0.1841\ t_0 - 2.9802$
60	0.19	17.2	R^2=0.9982
80	0.24	17.5	
100	0.30	17.8	
125	0.64	19.5	$\mu_m = 25.184\ X^{-1.2697}$
150	0.48	23.5	R^2=0.9046
200	0.35	25.7	
250	0.30	29.6	
300	0.28	36.3	
350	0.25	39.0	

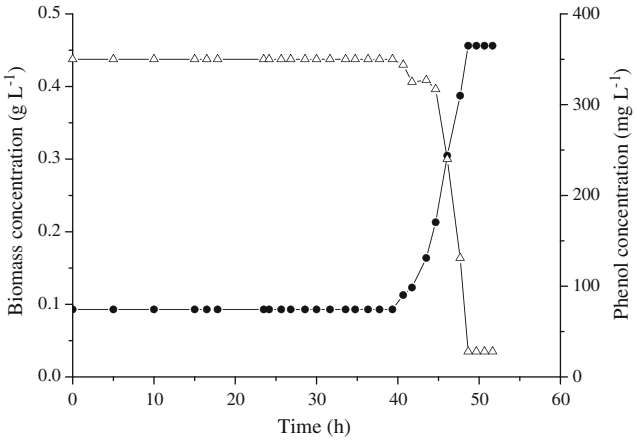

Fig. 4 Time-courses of phenol and cell concentrations for S_0= 350 mg L^{-1}

Table 2 Kinetic models for growth involving substrate inhibition

Model	Author	Model
1	Monod	$\mu = \frac{\mu_m S}{K_S+S}$
2	Haldane	$\mu = \frac{\mu_m S}{K_S+S+\left(\frac{S^2}{K_I}\right)}$
3	Luong	$\mu = \frac{\mu_m S}{K_S+S}\left(1-\left(\frac{S}{S_m}\right)\right)^n$

Table 3 Estimated values of parameters for the considered growth kinetic models

Model	μ_m (h^{-1})	K_s (mg L^{-1})	K_I (mg L^{-1})	S_m (mg L^{-1})	n	R^2
Monod	0.84	217.7	-	-	-	0.52
Haldane	0.66	76.1	205.4	-	-	087
Luong	1.04	153.2	-	540	0.9	0.91
Luong (n=1)	1.04	153.2	-	540	1	0.88

125 mg L^{-1} phenol. When the concentration of phenol (Fig. 3a) was <125 mg L^{-1}, the time of the lag phase t_0 increased linearly with increasing maximum specific growth rate; the opposite trend was observed for concentrations >125 mg L^{-1} (Fig. 3b), namely, the length of the lag phase t_0 (power form) increased with decreasing maximum specific growth rates. The corresponding results are shown in Table 1.

Experimental time-courses for biomass growth and phenol concentration (S_0=350 mg L^{-1}) are shown in Fig. 4. It is evident that even at the highest initial phenol concentration tested in our study, the concentration of phenol was the limiting substrate. This was expected owing to the composition of the culture medium (Materials and Methods), which showed that the other components are present in excess.

The influence of oxygen was not considered; it can be assumed that the aeration provided by shaking the flasks was adequate to ensure that oxygen concentration was not limiting, as indicated by the absence of linear growth (Fig. 1) as well as the distinct effect of the initial phenol amount on growth (Fig. 4). It can be therefore assumed that the growth rate of the mixed culture was only limited by the initial phenol concentration, as is shown in Fig. 4.

Various single substrate kinetic models for growth have been published as representive of the growth kinetics data for substrate limitation and inhibition. Three models were considered in this study (Table 2).

For all substrate concentration values, the three models were fitted to experimental data, and the model parameters evaluated using Statistica ver. 6.0 (StatSoft, Tulsa, OK) based on Windows XP (Microsoft, Redwood, WA). The kinetic parameter values as well as the correlation coefficient (R^2) for the three models are shown in Table 3.

Figure 5 illustrates the fit of experimental runs using the kinetic models considered in the study. This figure clearly shows that even if the Luong model overestimated

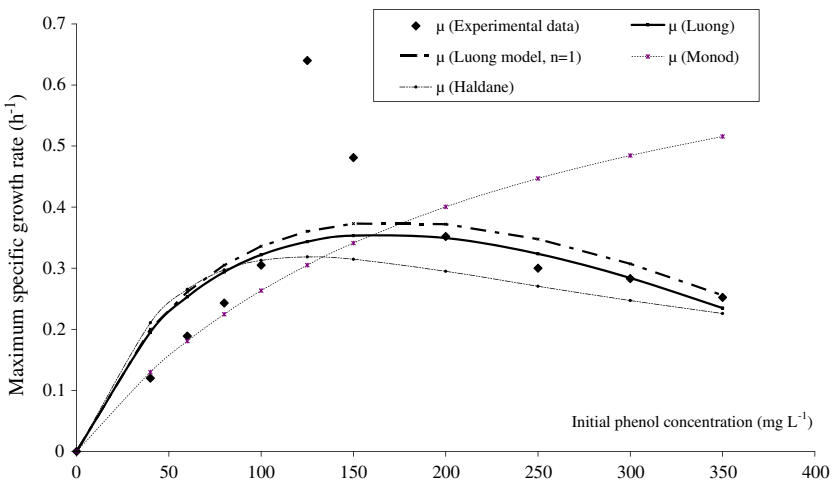

Fig. 5 Maximum specific growth rate μ_m as a function of the initial concentration S_0

the maximum specific growth rate, 1.044 h^{-1} versus 0.64 h^{-1}, for the experimental value, it describes the experimental data more accurately than the Haldane and the Monod models. The kinetic constants of the Luong equation are μ_m =1.04 h^{-1}, K_s = 153.2 mg L^{-1}, S_m = 540 mg L^{-1}, and n = 0.9, which fall within the range of values reported in the literature (Luong 1987; Pawlowsky and Howell 1973; Vijayagopal and Viruthagiri 2005a, b).

Conclusion

Phenol as the only carbon and energy source can be degraded by a mixed culture, as shown in our study for phenol concentrations between 40 and 350 mg L^{-1}. The time of the lag phase increased exponentially with the initial phenol concentration, with the maximum specific growth rate μ_m falling within the range of previously published values.

Based on the results of batch experiments carried out with phenol as the limiting substrate, at 30°C and pH=8, we fit three kinetic models to experimental data to describe growth, leading to the determination of kinetic parameters. The model of Luong appeared to be the most appropriate to fit the experimental data recorded during mixed culture for an initial phenol concentration ranging from 40 to 350 mg L^{-1}. The value of constants of the Luong equation, $\mu = (\mu_m S/(K_s + S))(1 - S/S_m)^n$, were μ_m=1.04 h^{-1}, K_s= 153.2 mg L^{-1}, S_m=540 mg L^{-1}, and n=0.9.

We therefore conclude that the mixed culture studied here can be successfully used to treat wastewater containing phenolic compounds, such as phenol.

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