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Short Communications

Batch kinetic studies in phenol biodegradation and comparison

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Pure cultures of *Pseudomonas putida*, *Nocardia* sp., *Bacillus circulans* and a mixed culture, isolated from mangrove forest soil, were used to study phenol biodegradation in the batch reactor. The aim was to determine the kinetics of phenol biodegradation by measuring the biomass growth rates and phenol concentration as a function of time in a batch reactor. The kinetic constants, specific growth rate (μ_m), inhibition coefficient for phenol (K_i) and half saturation coefficient for phenol (K_s) were determined using the Haldene equation [$\mu_m s/(K_s + S + (S^2/K_i)$]. The values obtained for kinetic constants are in the range of those published in literature for pure and mixed cultures degrading phenol. The length of the lag phase before the exponential growth phase increases linearly with phenol concentration. As compared to others, *P. putida* has the maximum specific growth rate both in the present study as well as in literature. Thus, selecting a pure culture of *P. putida* for phenol degradation could lead to higher efficiencies.

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Phenols are pollutants of wastewaters from oil refineries, chemical plants, explosives, resins and coke manufacture, coal conversion, pesticide and textile industries. Wastewaters containing phenol in the range of 5-500 mg L^{-1} are considered suitable for treatment by biological processes[1]. The literature on phenol biodegradation by mixed or pure cultures reports that many bacteria, but likely not all of them, metabolize phenol[2]. In the present work, *Pseudomonas putida*, *Nocardia* sp., *Bacillus circulans* and a mixed culture (isolated from mangrove forest soil) MC-1 were selected[3] as a known representative of the aerobic degraders of aromatics, especially phenol[4,5].

To describe substrate biodegradation, it is necessary to evaluate the relationship between the specific growth rate (μ) and the phenol concentration (S), as we know that phenol biodegradation by microbes has generally to be inhibited by phenol itself. The Haldane equation[6,7], presented below, has been frequently used to describe this degradation in pure or mixed cultures [2,8,9].

$$\mu = \frac{\mu_m S}{K_s + S + (S^2 / K_i)} \qquad \ldots(1)$$

where μ_m is the maximum specific growth rate, K_s the half-saturation coefficient and K_i the inhibition coefficient. The work described here is concerned with phenol-inhibitory growth kinetics of a pure culture of *P. putida, Nocardia* sp., *B. circulans* and a mixed culture. The aim of the study is to get kinetics data regarding the growth of a suspended microbial culture on phenol from batch experiments and to compare these results with those from other works using pure and mixed cultures.

Stock cultures of microorganisms, *P. putida*, *Nocardia* sp., *B. circulans* and a mixed culture (MC-1), were maintained by periodic subculture on nutrient agar slants, which were stored at 4°C. The microorganisms were grown on a mineral medium prepared with deionised water with phenol as the sole carbon and energy source [phenol, variable; KH_2PO_4, 420; K_2HPO_4, 375; $(NH_4)_2SO_4$, 244; NaCl, 15; $CaCl_2.2H_2O$, 15; $MgSO_4.7H_2O$, 50; $FeCl_3.6\ H_2O$, 5.4 mg L^{-1})]. The experiments were performed at 30±0.5°C in 250 ml Erlenmeyer flasks agitated and aerated by a reciprocal shaker at 150 oscillations per min. The concentration of microorganisms was determined by optical density measurement at 660 nm using a LS-164 double beam, UV-VIS digital, spectrophotometer. While phenol concentrations were measured at 510 nm by spectrophotometric method.

The kinetic parameters describing the growth of the free microbial culture on phenol were determined from batch growth experiments. A range of 50-250 mg L^{-1} was used as initial phenol concentration. 5 mL of inoculum (acclimatized) was added to each of the 250 mL shake flasks containing 95 mL of medium. Optical absorbance of the culture at 660 nm and the residual phenol concentration at 510 nm was measured every hour.

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The specific growth rate (μ) for each value of the initial phenol concentration (S_0) was determined in the exponential growth phase. Small volume of inoculum was used so that the substrate concentration could be assumed constant in this initial growth period. Following Manterio *et al*[2] who considered direct proportionality between biomass and optical density, μ was determined for each flask from the slope of linear semilogarithmic plots of optical density against time during the exponential growth phase, where μ was approximately constant.

The biodegradation of phenol has two phases, the lag and the growth phase. The influence of phenol concentration on the duration of the lag phase is shown for *P. putida* in Fig. 1. The length of the lag phase (t_0), was determined for each run as the time where the horizontal line corresponding to the initial optical density intersects the straight line drawn to determine the specific growth rate. Fig. 2 shows that t_0 increases with the increase in phenol concentration between 50 and 250 mg L^{-1}. However, high concentrations of phenol have an inhibitory effect on the microbial growth. This was also observed using mixed cultures[12,13]. Other workers using a pure culture of *P. putida*[14] observed that the length of lag phase increased exponentially with the increase in phenol concentration in the range 60-600 mg L^{-1}. In Fig. 2, this best-fit curve is also represented.

The variation of experimental specific growth rate as a function of phenol concentration and the fitted curve (Haldane model) is presented for *P. putida* in Fig. 3. The Haldane equation was fitted to the data using the non-linear least squares technique. Values of the kinetic constants obtained in this work are compared with other published data in Table 1. The values obtained in this work are in the range of literature values. *P. putida* has the maximum specific growth rate (μ_m), followed by *Nocardia* sp., MC-1 and *B. circulans* among the microbial systems listed. Hence, selecting a pure culture of *P. putida* for phenol degradation can lead to higher efficiencies as compared to mixed cultures. Further, the variation of kinetic parameters may be explained by the different history of inocula. Sokol and Howell[15] obtained greater values of μ for younger inocula exposed to lower phenol concentrations.

In the present work, authors have assumed that the growth rate of *P. putida*, *Nocardia* sp., *B. circulans* and MC-1 was only limited by phenol concentration. The influence of oxygen was not considered as it was understood that the aeration provided by shaking the

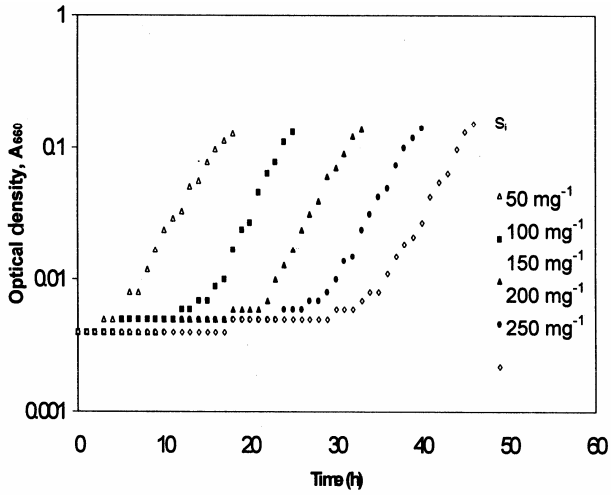

Fig. 1—Optical density at 660 nm as a function of time for *P. putida*

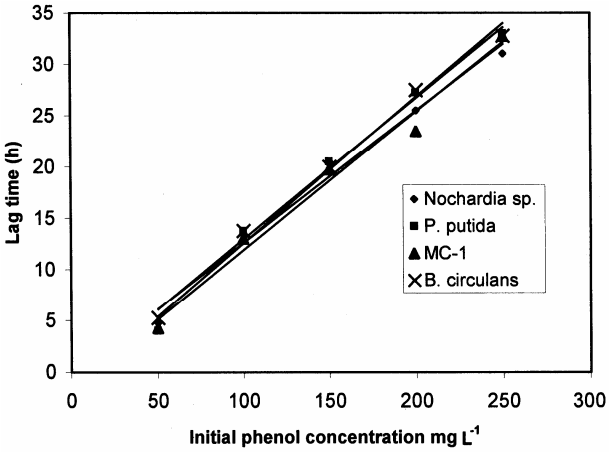

Fig. 2—Length of lag phase as a function of initial phenol concentration

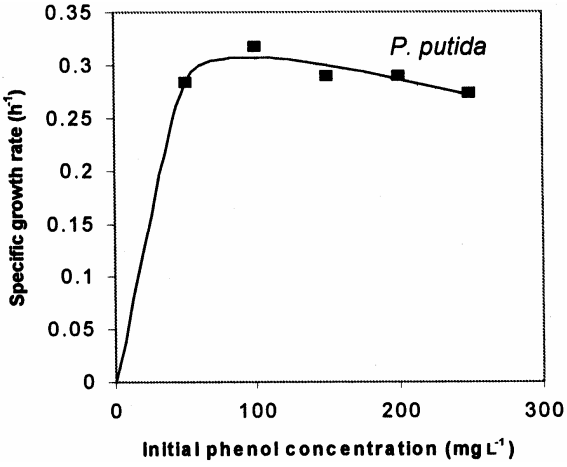

Fig. 3—Specific growth rate (μ) as a function of the initial phenol concentration
Phenol concentration (•); Model curve (—)

Table 1—Comparison of values of kinetic constants obtained in various studies

Culture	μ_m (h^{-1})	K_s (mg L^{-1})	K_i (mg L^{-1})	Ref.
*Pseudomonas putida**	0.4378	21.460	477.64	Present study
MC-1	0.3794	18.821	449.15	Present study
Nocardia sp.	0.4227	26.770	261.72	Present study
Bacillus circulans	0.2739	1.753	1743.09	Present study
Mixed	0.131-0.363	5-226	142-1199	1
T. cutaneum	0.464	1.66	380	2
P. putida DSM 50222	0.534	<1	470	10
Mixed	0.318	57.35	1503	13
P. putida DSM 548	0.26-0.9	0.16-0.62	14.9-19.4	15
Mixed	0.418	2.9	370	17
P. putida DSM 548	0.567	2.39	106	18
P. putida DSM 548	0.33-0.9	0.58-1.25	8.2-19.2	19
P. putida P71	0.569	18.5	99.4	20
Mixed	0.260	25.4	173	21
Mixed	0.223	5.86	934.5	21
Mixed	0.326	19.2	229.3	22
Mixed	0.365	10.95	113	23

flasks was sufficient to keep the oxygen concentration constant and not limiting. This means that μ was not described by a dual-substrate expression[16,17].

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