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Phenol biodegradation by mixed culture in batch reactor — Optimization of the mineral medium composition

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ABSTRACT

A new phenol-degrading microbial consortium with high biodegradation activity and high tolerance to phenol was isolated from activated sludge for hazardous wastewater treatment (Boumerdès, Algeria). Biodegradation kinetics was determined by monitoring biomass and phenol consumption. Mineral medium composition and especially nitrogen source concentration, as well as culture pH, were optimized. The temperature (30°C), the stirring velocity (200 rpm) and the phenol concentration (125 mg L^{-1}) were kept constant; while the initial pH was varied (5–9), and the mineral components were tested in the following concentration ranges: 0.25–2 g L^{-1} for $(NH_4)_2SO_4$, 1–4 g L^{-1} for KH_2PO_4 and NaH_2PO_4 and 0.05–0.2 g L^{-1} for $MgSO_4$. All experiments were carried out at a given initial bacterial concentration, 0.08 g L^{-1} (based on optical density determination, 0.079). Irrespective of the culture conditions, total phenol degradation (125 mg L^{-1}) was recorded for culture times ranging from 20.6 to 31.2 h. The optimal mineral medium concentrations were therefore, 1, 3, 3 and 0.1 g L^{-1} for $(NH_4)_2SO_4$, KH_2PO_4, NaH_2PO_4 and $MgSO_4$ respectively and the optimal pH was 8, leading to a specific growth rate of 0.64 h^{-1}. Higher maximum specific growth rate values were recorded during this work, if compared to those reported in the available literature, even those dealing with mixed culture. This result showed the relevance of the specific microbial consortium used.

Keywords: Biodegradation; Phenol; Microbial consortium; Batch kinetics

1. Introduction

Phenols are distributed either as natural or artificial mono-aromatic compounds in various environmental sites as major pollutants. Their existence in wastes from industrial processes such as oil refineries, coking plants, wastewater treatment plants, petroleum-based processing, and phenol resin industry manufacturing and plants, has been well established. Phenol and its derivatives are among the most frequently found pollutants in rivers, industrial effluents, and landfill runoff waters.

Biodegradation of phenol and its derivatives by microorganisms has been extensively studied and a large number of phenol-degrading bacteria have been isolated and characterized at the physiological and genetic level [1–4]. However, even if some information on microbial

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species with a high phenol tolerance and high metabolizing activity is available in the literature, there is still the need to isolate new phenol-degrading microorganisms that can aerobically grow at high phenol concentrations.

Several microbial strains have also demonstrated their ability to metabolize phenol as their sole source of carbon and energy, and include among others species *Alcaligenes faecalis* [5], *Aureobasidium pullulans* [6], *Candida tropicalis* [7,8], *Pseudomonas putida* [9,10], *Ralstonia taiwanensis* [11]. Biodegradation is typically limited by substrate inhibition leading to low growth rates at high phenol concentrations.

The main goal of this paper was to investigate the biodegradation of phenol by microbial consortium. In this aim, the mineral supplementation was optimized.

2. Materials and methods

2.1. Microorganisms cultivation

The microbial consortium used in this work was obtained from the activated sludge of Boumerdès station (Algeria). Stock cultures were stored at +4°C. The microorganisms were activated for 24 h at 30°C in a nutrient medium containing (g L^{-1}): peptone, 15, yeast extract, 3, sodium chloride, 6, and (D^+)-glucose, 1.

After 24 h, when cells were grown, the biomass was harvested by centrifugation. The microorganisms collected after centrifugation (3000 rpm for 30 min) were suspended in NaCl 0.5 % and re-centrifuged. After the third washing, the microorganisms collected after centrifugation were re-suspended again in NaCl 0.5% and the microbial concentration was deduced from turbidimetric measurements. In this aim, after OD measurement at 600 nm (Vis spectrophotometer – HACH DR2800), the OD value was 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

$$x\ (\text{g L}^{-1}) = 1.044 \times \text{OD600} \quad (1)$$

Specific growth rate was determined in the exponential growth phase [12–15]. For each flask, it was determined from the time-course of the linear semi logarithmic plot of cell concentration during the exponential growth phase, namely when the specific growth rate was approximately constant [16].

The biomass on substrate yield $Y_{X/S}$ was deduced from initial and final concentrations, namely

$$Y_{X/S} = \frac{x_f - x_i}{s_i - s_f} \quad (2)$$

with x_i and x_f initial and final biomass concentrations, and s_i and s_f initial and final phenol concentrations.

2.2. Biodegradation experiments

For an OD value of the adapted cells in the range 2.7–2.9, an aliquot of the culture was centrifuged at 3000 rpm for 30 min. To wash the biomass, it was re-suspended in NaCl 0.5% and centrifuged. The cells (1 ml) were then transferred and inoculated in Erlenmeyer flasks (250 mL) to yield an initial OD of 0.078, and containing 100 mL of culture medium containing nitrogen source ((NH4)2SO4) and the following mineral salt supplementation (MSS), namely NaH_2PO_4, KH_2PO_4 and $MgSO_4$ at the required concentrations, and 125 mg L^{-1} of phenol. The cells were cultivated at 30°C and 200 rpm. Samples were withdrawn at suitable time intervals and the biomass concentration was indirectly monitored by means of turbidimetric (OD) measurements as described above; while phenol was colorimetrically estimated using a Vis spectrophotometer (HACH DR2800) according to the method previously described by Yang and Humphrey [17] and based on rapid condensation with 4-aminoantipyrine followed by oxidation with alkaline potassium ferricyanide and absorbance read at 510 nm.

3. Results and discussion

3.1. Optimal salt concentration

The effect of NaH_2PO_4, KH_2PO_4 and $MgSO_4$ concentrations on phenol degradation by the microbial consortium was examined in Table 1 and the corresponding time-courses are displayed for $MgSO_4$ in Fig. 1.

Complete degradation of phenol (125 mg L^{-1}) by the mixed cultures was achieved in a culture time ranging from 20.6 to 33.2 h. The lowest biodegradation time (20.6 h) was recorded for NaH_2PO_4 concentration (Table 1) in the range 2–4 g. L^{-1}, for 3 g L^{-1} KH_2PO_4 (Table 1) and for 0.1 g L^{-1} $MgSO_4$ (Fig. 1). It should be noted a time lag of at least 10 h irrespective of the considered culture conditions.

Table 1 also shows a low impact of the mineral medium supplementation on the biomass on substrate yield. Contrarily, a noticeable effect on the maximum value of the specific growth rate can b observed, and a maximum μ_{max} value of 0.38 h^{-1} was recorded for the following salt concentrations, 3 g L^{-1} NaH_2PO_4 or KH_2PO_4 or 0.1 g L^{-1} $MgSO_4$; these concentrations can be considered as the optimal mineral salt supplementation.

The analysis of the results shows that:

- The optimal concentration of 3 g L^{-1} NaH_2PO_4 (Table 1) was in the range of concentration values reported in the available literature. Indeed, for phenol biodegradation studies Luo et al. [18] as well as Nakano et al. [19] considered 2.544 and 1 g L^{-1} of NaH_2PO_4 respectively to supplement their culture medium. To biodegrade chlorophenols (2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol),

Table 1
Maximum specific growth rate μ_{max} and biomass on substrate yield $Y_{X/S}$ values for the various culture conditions tested

Parameter	Salt (g L^{-1})	μ_{max} (h^{-1})	$Y_{X/S}$	Degradation time (h)	Lag time (h)
$[NaH_2PO_4]^a$	1	0.22	0.0011	21.8	10.0
	2	0.31	0.0012	20.6	10.0
	3	0.38	0.0012	20.6	10.0
	4	0.22	0.0012	20.6	10.0
$[KH_2PO_4]^b$	1	0.36	0.0011	30.2	22.7
	2	0.33	0.0012	31.2	22.7
	3	0.38	0.0012	20.6	10.0
	4	0.37	0.0011	31.2	22.7
$[MgSO_4]^c$	0.05	0.27	0.0009	24.7	10.0
	0.1	0.38	0.0012	20.6	10.0
	0.15	0.31	0.0012	24.7	10.0
	0.2	0.28	0.0011	25.6	10.0

[a]$[(NH_4)_2SO_4]$ = 1 g L^{-1}; $[KH_2PO_4]$ = 3 g L^{-1}; $[MgSO_4]$ = 0.1 g L^{-1}.
[b]$[(NH_4)_2SO_4]$ = 1 g L^{-1}; $[NaH_2PO_4]$ = 3 g L^{-1}; $[MgSO_4]$ = 0.1 g L^{-1}.
[c]$[(NH_4)_2SO_4]$ = 1 g L^{-1}; $[NaH_2PO_4]$ = 3 g L^{-1}; $[KH_2PO_4]$ = 3 g L^{-1}.

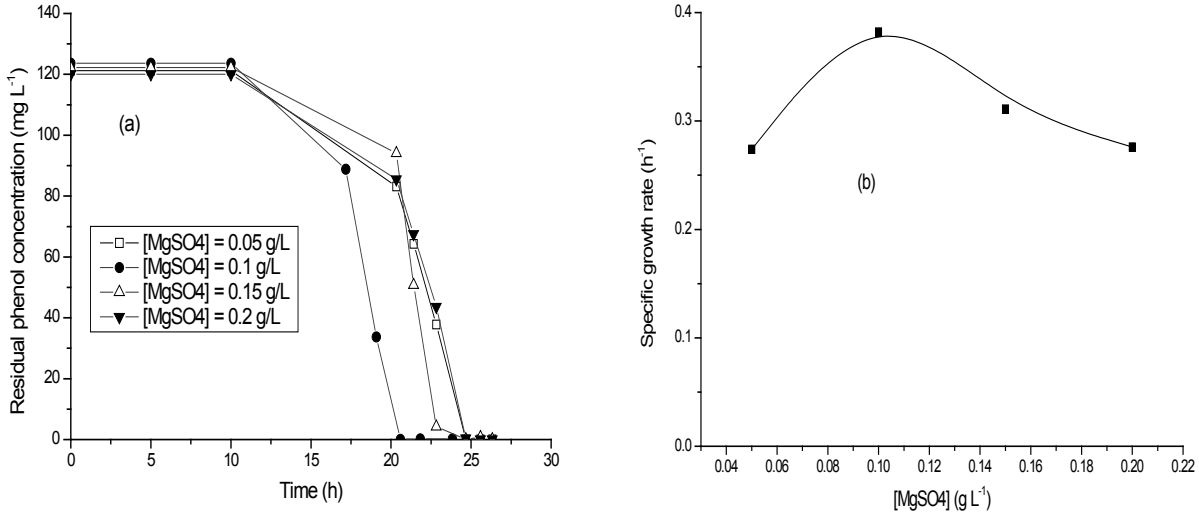

Fig. 1. (a) Time-courses of the residual phenol concentration for different initial $MgSO_4$ concentration, (b) specific growth rate versus initial $MgSO_4$ concentration ($[(NH_4)_2SO_4]$ = 1 g L^{-1}; $[NaH_2PO_4]$ = 3 g L^{-1}; $[KH_2PO_4]$ = 3 g L–1; [Phenol] = 125 m g L^{-1}; Temperature = 30°C; stirring velocity = 200 rpm; pH = 7).

4 g L^{-1} of NaH_2PO_4 were used in mineral salt medium by Zilouei et al. [20].

- dos Santos et al. [21] used 4.3 g L^{-1} of KH_2PO_4 in mineral salt medium for phenol biodegradation by Aureobasidium pullulans FE13 isolated from industrial effluents, namely a higher amount than the optimal value found in this work (3 g L^{-1} – Table 1).
- -The same optimal (Fig.1b) value of $MgSO_4$ concentration (0.1 g L^{-1}) in mineral salt medium was considered by Zhao et al. [22] to biodegrade phenol.

3.2. Optimal nitrogen source supplementation

Fig. 2 shows the effect of $(NH_4)_2SO_4$ concentration on phenol degradation. It should be noted that the optimal mineral salt supplementation was considered for these

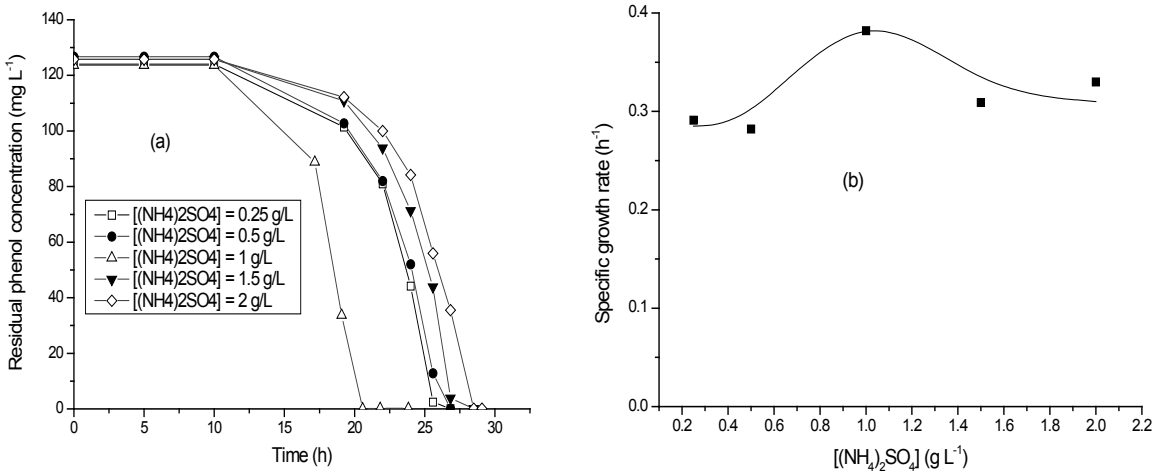

Fig. 2. (a) Time-courses of the residual phenol concentration for different initial $(NH_4)_2SO_4$ concentration, (b) specific growth rate vs. initial $(NH_4)_2SO_4$ concentration ([NaH_2PO_4] = 3 g L^{-1}; [KH_2PO_4] = 3 g L^{-1}; [$MgSO_4$] = 0.1 g.L^{-1}; [Phenol] = 125 mg L^{-1}; Temperature = 30°C; stirring velocity = 200 rpm; pH = 7).

batch cultures as well as a neutral pH 7. Irrespective of the culture conditions, total phenol biodegradation (125 mgL^{-1}) was recorded within 28.5 h (Fig. 2a); the shortest time for phenol biodegradation was 20.6 h recorded for an optimal concentration of 1 g L^{-1}.

The evolution of cell concentration shows that the nitrogen source concentration has no noticeable effect on the biomass on substrate yield (not shown) and that the lag time was at least 10 h. Fig. 2b shows the effect of the nitrogen source concentration on maximum specific growth rate; its maximum value was 0.38 h^{-1} for 1 g L^{-1} $(NH_4)_2SO_4$.

3.3. Optimal pH

Batch cultures of microbial consortium were conducted with the optimal mineral salt supplementation, 1 g L^{-1} $(NH_4)_2SO_4$, 125 mg L^{-1} of phenol and for an initial pH ranging from 5 to 9.

Time-courses of the residual phenol and biomass concentrations for different value of initial pH were displayed in Fig. 3. Irrespective of the initial pH, total phenol removal (125 mg L^{-1}) was recorded after a culture time ranging from 26.7 to 24.2 h; this latter time was recorded for a pH value of 8 (Fig. 3a).

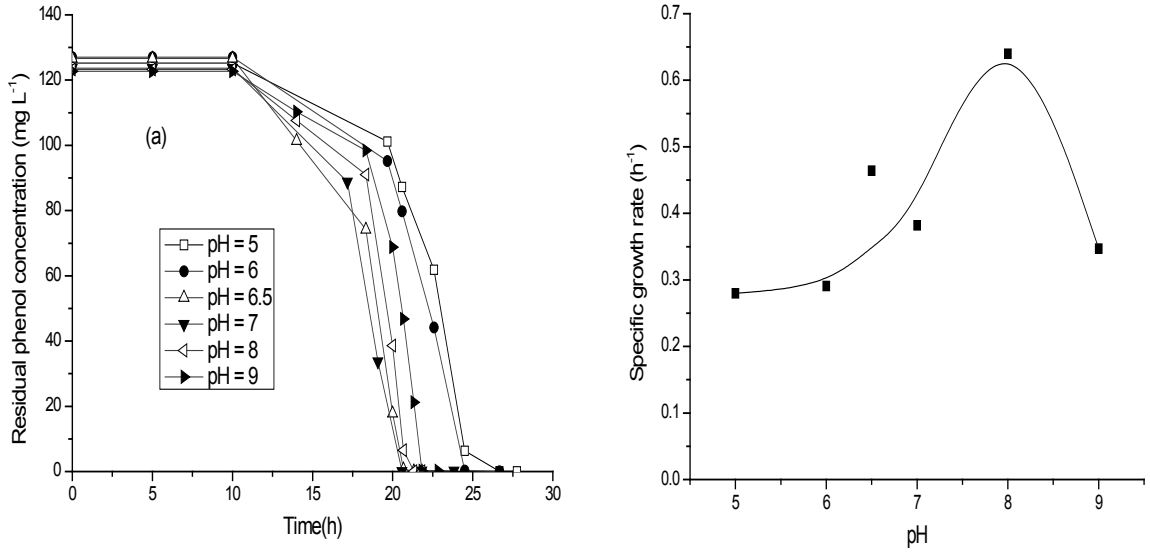

Fig. 3. (a) Time-courses of the residual phenol concentration for different value of initial pH, (b) specific growth rate versus different value of initial pH ([NaH_2PO_4] = 3 g.L^{-1}; [KH_2PO_4] = 3 g.L^{-1}; [$MgSO_4$] = 0.1 g.L^{-1}; [$(NH_4)_2SO_4$] = 1 g.L^{-1}; [Phenol] = 125 mg.L^{-1}; Temperature = 30°C; stirring velocity = 200 rpm).

The evolution of cell concentration shows that the lag time was in all cases between 10 and 14 h. In addition, and similarly to the other examined parameters, salt and nitrogen addition, no noticeable effect of the pH on $Y_{X/S}$ was observed (not shown). Fig. 3b shows that the maximum specific growth rate value was 0.64 h^{-1}, recorded for pH 8. This optimal pH value was in agreement with other workers in the field (Ho et. al. [2], Qiu et al. [23] and Ying et al. [24]) who had obtained the same optimal pH for phenol and p-nitrophenol removal.

If compared to those reported in the available literature, higher maximum specific growth rate values were recorded during this work. Indeed, the values reported in the available literature even those dealing with mixed culture are in the range 0.13–0.36 h^{-1} [16,17, 25–29]. This result shows the relevance of the specific microbial consortium used.

4. Conclusions

The following conclusions can be drawn from growth kinetics of microbial consortium and the related phenol degradation time-courses:

- The considered microbial consortium has a high biodegradation activity, since the maximum growth rate was 0.64 h^{-1}.
- Irrespective of the culture conditions, total phenol degradation (125 mg L^{-1}) was recorded within culture time ranging from 20.6 to 31.2 h.
- The optimal mineral medium supplementation (g L^{-1}) was 1, 3, 3 and 0.1 g L^{-1} for $(NH_4)_2SO_4$, KH_2PO_4, NaH_2PO_4 and $MgSO_4$, respectively; and the optimal pH value of pH was 8.

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