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Kinetics of aerobic phenol biodegradation by the acidophilic and hyperthermophilic archaeon *Sulfolobus solfataricus* 98/2

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ABSTRACT

Biodegradation of 51–745 mg l^{-1} of phenol by a well-acclimatized strain of *Sulfolobus solfataricus*, a thermoacidophilic archaeon, was studied in batch experiments at 80 °C and pH 3.2. Phenol inhibited growth and specific degradation rates (μ and q_S). Fitting the experimental growth data with the Haldane model gave the following kinetic parameters: $\mu^* = 0.094\ h^{-1}$, $K_S = 77.7$ mg l^{-1}, $K_i = 319.4$ mg l^{-1} ($R^2 = 0.950$). The true μ_{max}, calculated from μ^*, was 0.047 h^{-1}. A volumetric degradation rate (V_{max}) was calculated by fitting the phenol consumption data with the Gompertz model. The value of V_{max} increased with initial phenol concentration (S_i) up to 14.4 mg l^{-1} h^{-1}. The q_S values, calculated from V_{max}, were fitted with the Haldane equation, yielding q_{Smax} of 0.110 g g^{-1} h^{-1}. The yield factor ($Y_{X/S}$) depends on S_i and reached a maximum of 0.83 g g^{-1} at $S_i = 93$ mg l^{-1}.

S. solfataricus 98/2 displayed low μ_{max} and q_{Smax} but a good tolerance to phenol (fairly high K_i, K_i', high $Y_{X/S}$). This ability to grow on and degrade phenol (93 mg l^{-1} < optimal S_i < 175 mg l^{-1}) at high temperature and low pH is unique and may be useful for removing phenol from hot acidic contaminated effluents. Other possible application could lie in the production of the enzymes involved in the key steps of phenol degradation provided the cloning of the enzymes-related genes in fast-growing mesophiles.

1. Introduction

Phenol is an organic pollutant present in wastewater from various industries such as refining, coking, coal processing and petrochemicals production. In some industrial effluents, it can reach concentrations up to 6.8 g l^{-1}, while the European-Union recommendations set the upper limits at 0.5 μg l^{-1} for potable water and 0.5 mg l^{-1} for wastewater emissions [1]. Most of the removal strategies for phenol-contaminated waters involve physical and (or) chemical technologies. However, microbial treatments leading to a complete mineralization have been shown to be, at the same time, economical and versatile, and have been widely studied in the last two decades [2]. Most reports concern single bacterial strains of the *Pseudomonas* [3–9] or *Alcaligenes* [10] genus, mixed bacterial consortia [11–14], or yeast genera [15,16], with maximum specific growth rates (μ_{max}) in the range of 0.036-0.385 h^{-1} (Table 1), specific degradation rates (q_S) of 0.057-0.940 g g^{-1} h^{-1} (Table 2) and yield factors ($Y_{X/P}$) of 0.44-0.90 g g^{-1} (Table 2), depending on strains and culture conditions.

Phenol is recognized as an inhibitory substrate at relatively low concentrations (100 mg l^{-1}) and is a convenient model for studying the kinetics of aromatic molecule degradation [17]. Different hypotheses have been proposed to explain the inhibition: *in vivo* inhibition of phenol-hydrolase activities in a *Ralstonia* strain [18], or possible effects on membrane functionality and subsequent increase in the energy needed to maintain cell-membrane integrity [3]. Microbial growth on this substrate has been successfully described by substrate-inhibition models. Amongst these, the Haldane equation has provided satisfactory correlations with experimental data (Table 1).

Although many phenol wastewaters are hot, only a few reports have dealt with phenol biodegradation by thermophilic [19–21] or hyperthermophilic [22,23] microorganisms. However, hyperthermophilic microorganisms can be a source of rare and robust biocatalysts with potential biotechnological applications [24], and their advantages compared with mesophilic ones have been described elsewhere [25]. For example, heat- and acid-stable amylase, cyclomaltodextrinase and endoglucanase from *Alicyclobacillus acidocaldarius* and glycosyl hydrolases from *Sulfolobus solfataricus* have been purified and characterized for further possible expression of the corresponding genes in mesophilic hosts [24,26]. In the field of thermostable enzymes, if many research works – and even applications – have dealt with heat-stable DNA polymerases or hydrolases such as proteases, amylases, cellulases or lipases, very few works on oxygenases have been reported up till now [27].

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Nomenclature

k	fitting parameter of the Gompertz model (h^{-1})
K_S, K_i	fitting parameters of the Haldane model applied to growth rate ($mg\,l^{-1}$)
K_S, K'_i	fitting parameters of the Haldane model applied to specific degradation rate ($mg\,l^{-1}$)
q_S	specific degradation rate ($g\,g^{-1}\,h^{-1}$)
q_{Smax}	maximum specific degradation rate ($g\,g^{-1}\,h^{-1}$)
S	phenol concentration ($mg\,l^{-1}$)
S_i	initial phenol concentration ($mg\,l^{-1}$)
S_m	phenol concentration at which $\mu = \mu_{max}$ ($mg\,l^{-1}$)
S'_m	phenol concentration at which $q_S = q_{Smax}$ ($mg\,l^{-1}$)
t_{opt}	time of maximum phenol degradation rate (h)
V_{max}	maximum volumetric rate of phenol degradation ($mg\,l^{-1}\,h^{-1}$)
X, X_{opt}	biomass concentration ($mg\,l^{-1}$)
$Y_{X/S}$	yield factor ($g\,g^{-1}$)
Greek symbols	
α, β	fitting parameters of the Gompertz model ($mg\,l^{-1}$)
μ	growth rate (h^{-1})
μ^*	fitting parameter, apparent maximum growth rate (h^{-1})
μ_{max}	true maximum growth rate (h^{-1})

However, oxygenases are involved in aerobic biodegradation of aromatic molecules and should be, in the future, an increasing field of research. As a matter of fact, some preliminary works on phenol have been done with *Sulfolobus*. For example, aerobic phenol biodegradation in Erlenmeyer flask by *S. solfataricus* (P2 strain) has been reported [22]. In the same way, *S. solfataricus* 98/2 strain was shown to grow on phenol ($365\,mg\,l^{-1}$) as sole carbon and energy source in a controlled batch fermentor [23]. The complete transformation of phenol into biomass and CO_2, under non-limiting oxygen conditions, showed that this microorganism displayed powerful oxygenase activity, like phenol hydroxylase or catechol dioxygenase [22,23]. However, none of these works have ever precisely described the kinetic aspects of phenol degradation. Finally, the complete genome of *S. solfataricus* 98/2 has been totally sequenced [28] which may allow the production of the proteins involved in the key steps of phenol degradation by fast-growing mesophiles for further industrial applications.

The aim of this work was to use the Haldane model to determine growth kinetics parameters (μ_{max}, K_S, K_i) for the thermoacidophilic archaeon *S. solfataricus* 98/2 grown in batch cultures at different initial phenol concentrations (S_i). For each concentration, the volumetric (V_{max}) and specific (q_S) degradation rates, as well as the yield factor ($Y_{X/S}$), were calculated and the whole set of data then compared with results reported in the literature.

2. Material and methods

2.1. Microorganism and medium

The thermoacidophilic *S. solfataricus* 98/2 strain was used in this study. It was kept at −80 °C and reactivated in a standard mineral medium (see composition given below) [29]. The strain was previously adapted to phenol through repeated batches with phenol as sole carbon and energy sources at a concentration of $400\,mg\,l^{-1}$, previously shown to be tolerated by the microorganism [23]. It was grown at 80 °C, on a standard mineral medium with the following composition ($g\,l^{-1}$): 1.3 $(NH_4)_2SO_4$, 0.28 KH_2PO_4, 0.25 $MgSO_4{\cdot}7H_2O$, 0.07 $CaCl_2{\cdot}2H_2O$, 0.02 $FeCl_3{\cdot}6H_2O$, and ($mg\,l^{-1}$): 1.8 $MnCl_2{\cdot}4H_2O$, 4.5 $Na_2B_4O_7{\cdot}10H_2O$, 0.22 $ZnSO_4{\cdot}7H_2O$, 0.05 $CuCl_2{\cdot}2H_2O$, 0.03 $NaMoO_4{\cdot}2H_2O$, 0.03 $VOSO_4{\cdot}2H_2O$, 0.01 $CoSO_4{\cdot}7H_2O$ The pH was adjusted to 3.2.

2.2. Experimental

Cultures were performed by adding approx. 10 ml of fresh inoculum (OD_{600} ∼1) to 90 ml of mineral medium in 500 ml Schott bottles. Phenol ($20\,g\,l^{-1}$) was supplied to reach concentrations ranging from $51\,mg\,l^{-1}$ to $745\,mg\,l^{-1}$. Schott bottles, hermetically closed with a high-temperature-resisting plastic cap, were then placed on a rotating shaker at 150 rpm and 80 °C. Each experiment was duplicated. Samples were withdrawn periodically to determine biomass production and phenol consumption. Every time the bottle was opened, the atmosphere was renewed, maintaining a sufficient aeration for the culture, since a recent study demonstrated that the range of optimal oxygen concentration for *S. solfataricus* grown on glucose was broad, between 1.5 and 24% oxygen in the gas phase [30]. Oxygen is a key parameter since the degradation of phenol requires high amounts of oxygen (theoretical molar phenol-oxidation ratio = 7 mol O_2 per mol of phenol). In order

Table 1
Fitting parameters (μ^*, K_S, K_i) for Haldane equation and calculated parameters (S_m, μ_{max}) of various microorganisms grown on phenol (S_i).

Microorganisms	S_i[a] ($mg\,l^{-1}$)	μ^* (h^{-1})	K_S ($mg\,l^{-1}$)	K_i ($mg\,l^{-1}$)	S_m[b] ($mg\,l^{-1}$)	μ_{max}[c] (h^{-1})	References
Pseudomonas putida Q5	600	0.419	7.09	221	39.6	0.308	[3]
P. putida F1	200	0.051	18	430	88.0	0.036	[5]
P. putida CCRC 14365	400	0.245	12.1	1184	119.7	0.204	[6]
P. putida MTCC 1194	400	0.109	53.2	148.6	88.9	0.050	[7]
P. putida MTCC 1194	1000	0.305	36.33	129.8	68.7	0.148	[8]
P. putida ATCC 17484	700	0.534	<1	470	nc	nc	[17]
11 isolated *Pseudomonads*	900	[0.231–0.931][d]	–	[187–1236][d]		–	[9]
Alcaligenes faecalis	1400	0.15	2.22	245.4	23.3	0.126	[10]
Ewingella americana	1000	0.290	5.16	1033.7	73.0	0.254	[33]
Mixed microbial population	700	0.309	74.6	648	219.7	0.184	[13]
Mixed microbial population	800	0.308	44.9	525	153.5	0.194	[14]
Trichosporon cutaneum R57	500	0.420	110	380	204.4	0.202	[15]
Candida tropicalis	400	0.643[e]	7.1	185	36.2	0.385	[11]
C.tropicalis	2000	0.48	11.7	207.9	49.3	0.325	[16]
Sulfolobus solfataricus 98/2	745	0.094	77.7	319.4	157.5	0.047	This study

[a] Maximum value.
[b] Calculated according to Eq. (2).
[c] Calculated according to Eq. (3).
[d] Calculated with the Aiba–Edwards model [9].
[e] Given by $\mu^* = k\mu_{max}$ ($k = 1.67\,g\,g^{-1}$).