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ORIGINAL PAPER

Phenol biodegradation by the thermoacidophilic archaeon *Sulfolobus solfataricus* 98/2 in a fed-batch bioreactor

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Abstract Toxic at low concentrations, phenol is one of the most common organic pollutants in air and water. In this work, phenol biodegradation was studied in extreme conditions (80°C, pH = 3.2) in a 2.7 l bioreactor with the thermoacidophilic archaeon *Sulfolobus solfataricus* 98/2. The strain was first acclimatized to phenol on a mixture of glucose (2000 mg l^{-1}) and phenol (94 mg l^{-1}) at a constant dissolved oxygen concentration of 1.5 mg l^{-1}. After a short lag-phase, only glucose was consumed. Phenol degradation then began while glucose was still present in the reactor. When glucose was exhausted, phenol was used for respiration and then for biomass build-up. After several batch runs (phenol < 365 mg l^{-1}), specific growth rate (μ_X) was 0.034 ± 0.001 h^{-1}, specific phenol degradation rate (q_P) was 57.5 ± 2 mg g^{-1} h^{-1}, biomass yield ($Y_{X/P}$) was 52.2 ± 1.1 g mol^{-1}, and oxygen yield factor $\left(Y_{X/O_2}\right)$ was 9.2 ± 0.2 g mol^{-1}. A carbon recovery close to 100% suggested that phenol was exclusively transformed into biomass (35%) and CO_2 (65%). Molar phenol oxidation constant $\left(Y_{O_2/P}\right)$ was calculated from stoichiometry of phenol oxidation and introducing experimental biomass and CO_2 conversion yields on phenol, leading to values varying between 4.78 and 5.22 mol mol^{-1}. Respiratory quotient was about 0.84 mol mol^{-1}, very close to theoretical value (0.87 mol mol^{-1}). Carbon dioxide production, oxygen demand and redox potential, monitored on-line, were good indicators of growth, substrate consumption and exhaustion, and can therefore be usefully employed for industrial phenol bioremediation in extreme environments.

Keywords *Sulfolobus solfataricus* · Phenol · Kinetics · Biodegradation · Yields

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Introduction

Phenol is a widespread pollutant found in liquid and gaseous pharmaceutical, electronic and chemical-industry effluents, such as polycarbonate resins, oil refineries, and coke ovens. In 2008, phenol represented a 5%-a-year growth market, and the price for phenol was about 1100 US$/ton for a total worldwide production estimated at around 10.7 million tons (Busca et al. 2008).

Phenol can be degraded by a large number of mesophilic microorganisms, including yeasts from the *Candida* genus (Adav et al. 2007) and bacteria, mainly from *Pseudomonas* (Onysko et al. 2000; Viggor et al. 2008; Shourian et al. 2009) and *Alcaligenes* (Müller and Babel 1996; Bai et al. 2007) genera (Table 1).

Many phenol-containing wastewaters present high temperatures, prompting steady research into thermophilic bacteria over the last three decades. A *Bacillus stearothermophilus* strain isolated from a marine brine sample and displaying halophilic (10% NaCl) and thermophilic (50°C) characteristics degraded phenol at up to 1000 mg l^{-1} (Yanase et al. 1992), while a closely-related *B. stearothermophilus* strain isolated from Icelandic hot springs and grown at 65–70°C was shown to degrade 470 mg l^{-1} of phenol (Mutzel et al. 1996). In a 2-l bioreactor, growth rates (μ_X) up to 2.8 h^{-1} were obtained with *B. thermoleovorans* cultivated at 65°C maintaining a very low phenol concentration (15 mg l^{-1}) (Feitkenhauer et al. 2001). These values are much higher than those commonly reported for mesophiles (Table 1).

Hyperthermophiles are characterized by a growth temperature ranging from 80 to 110°C. Belonging mostly to the Archaea domain, they exist in many phylogenetically different groups and their biotopes (submarine hydrothermal areas, solfatara) exist since the Archaean period (Stetter et al. 1990). Moreover, thermoacidophilic microorganisms are reported as potential sources of rare and robust extracellular heat and acid-stable biocatalysts for biotechnological applications (Bertoldo et al. 2004). Table 2 summarizes selected parameters of the growth of *Sulfolobus* species cultivated on different substrates.

Studies on phenol biodegradation by hyperthermophilic archaea have only reported on two strains: P2 and 98/2, both of the acidic archaeon *Sulfolobus solfataricus*. The P2 strain, isolated from solfatara in Italy, was shown to degrade up to 750 mg l^{-1} of phenol at 80°C in Erlenmeyer flask cultures (Izzo et al. 2005). In contrast, the 98/2 strain, from Yellowstone National Park (USA), is reportedly unable to grow on phenol, which is surprising given the homologous putative aromatic hydrocarbon oxygenase gene cluster in both strains (Chae et al. 2007).

Due to the difficulties involved in working with very-high-temperature bioreactors for large periods, few studies to date have addressed the use of hyperthermophiles in equipped fermentors. Some have cited difficulties with continuous measurements of pH and redox potential (Nemati et al. 2000) or with pH and oxygen supply regulations (Nicolaus et al. 1991) due to the harshness of the environment for probes, while others have cited problems of evaporation of liquid medium (Park and Lee 1997; Worthington et al. 2003) and *a fortiori* relatively volatile

Table 1 Selected kinetic parameters and biomass yields for phenol biodegradation by mesophilic and thermophilic microorganisms

Microorganisms	Phenol (mg l^{-1})	Temp (°C)	μ_X (h^{-1})	q_P (mg g^{-1} h^{-1})	$Y_{X/S}$ (g mol^{-1})	References
Candida tropicalis	1000	30	0.385	390	93	Adav et al. 2007
Pseudomonas putida	300	25	0.176	429	38.5	Onysko et al. 2000
Pseudomonas putida	235	30	0.931	ng	51.2	Viggor et al. 2008
Pseudomonas fluorescens	235	30	0.231	ng	44.1	Viggor et al. 2008
Alcaligenes eutrophus	C-limiting	30	0.28	373	70.5	Müller et al. 1996
Alcaligenes faecalis	300	30	0.067	164	76.4	Bai et al. 2007
Bacillus stearothermophilus	94	65	0.20	ng	ng	Mutzel et al. 1996
Bacillus thermoleovorans	300	65	0.96	1041	86.5	Feitkenhauer et al. 2001

ng Not given

Table 2 Growth rates and conversion yields for some *Sulfolobus* species grown on different substrates

Microorganisms	Substrate	Concentration (mg l^{-1})	Temp (°C)	μ_X (h^{-1})	$Y_{X/S}$ (g mol^{-1})	References
S. metallicus	Pyrite	30000	68	0.025	–	Nemati et al. 2000
S. solfataricus P2	Glucose	2000	80	0.069	66.5	Simon et al. 2009
S. solfataricus ATCC 49115	Glucose	2000	88	0.166	55.8	Nicolaus et al. 1991
S. solfataricus DSM 1616	Glucose	<10000	70	0.067	96.5	Sönmezisik et al. 1998
S. solfataricus P2	Phenol	375	80	0.021	54.5	Izzo et al. 2005

substrates such as phenol due to the high temperatures used. As with any metabolic reactions in aerobic microorganisms, oxygen availability is a key parameter. Given the low solubility of oxygen in water at high temperature (3 mg l^{-1} at 80°C) and the high oxygen demand for aerobic phenol degradation $\left(7\ mol_{O_2}\ mol_{phenol}^{-1}\right)$, attention has focused on oxygen availability (Nikakhtari and Hill 2005; Ali et al. 1998), although dissolved oxygen concentration (DOC) measurement and control at high temperatures has not yet been reported in this process.

The first aim of this work was to determine whether *S. solfataricus* 98/2—genetically more stable than the P2 strain—was able to use phenol as sole carbon and energy source, after growth on glucose (glucose-grown cells) and repeated batch-runs on phenol (phenol-grown cells). The second aim was to run a 2.7-l bioreactor experiment to profile growth, substrate and oxygen consumption, carbon dioxide production, and the time-course pattern of redox potential for both glucose-grown and phenol-grown cells cultivated in conditions of controlled pH (3.2), temperature (80°C), and DOC (1.5 mg l^{-1}). The kinetic and respirometric rates and yields of phenol degradation were calculated, and carbon and oxygen balances were analyzed in detail.

Materials and methods

Strain and medium

Sulfolobus solfataricus 98/2 was provided by Pr. Paul Blum from the University of Nebraska (Rolfsmeier and Blum 1995). It was kept at −80°C and reactivated at 80°C on solid medium containing standard mineral medium (see composition given below) (Brock et al. 1972), added with sucrose (2 g l^{-1}), yeast extract (1 g l^{-1}) and Gelrite (10 g l^{-1}).

The standard mineral medium contained (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 $CuCl_2{\cdot}2H2O$, 0.03 $NaMoO_4{\cdot}2H_2O$, 0.03 $VOSO_4{\cdot}2H_2O$, 0.01 $CoSO_4$. pH was adjusted to 3.2 with 5 N H_2SO_4. Glucose (200 g l^{-1}) or phenol (20 g l^{-1}) was added before inoculation to reach concentrations of 2000 mg l^{-1} for glucose and 94–365 mg l^{-1} for phenol, which is a concentration widely reported as non-inhibitory for thermophilic (Mutzel et al. 1996; Feitkenhauer et al. 2001) or hyperthermophilic microorganisms (Izzo et al. 2005).

Experimental set up

Sulfolobus solfataricus 98/2 was batch-cultivated in a 2.7-l double-jacketed glass bioreactor (FairmenTec, France) with a working volume of 1.8 l. The bioreactor was autoclaved ex situ at 121°C for 20 min in a Federagi FVG apparatus. Mixing was driven by two axial impellers, and the fermentor vessel was equipped with probes monitoring temperature (Prosensor pt 100, France), pH (InPro 3253, Mettler Toledo, Switzerland), redox potential (InPro 3253, Mettler Toledo, Switzerland) and DOC (InPro 6800, Mettler Toledo, Switzerland). The probes were calibrated before and verified after the experiments to validate the parameters measured. The inlet gas stream (mixture of air and nitrogen) was injected through a nozzle immersed in the bioreactor. The steam in the outlet gas stream was condensed into a glass exhaust water cooler temperature-controlled at 4°C by a cooling bath equipped with a pump (Julabo F25, France) to prevent evaporation (water and phenol vapour condensates were returned to the cultivation vessel). On the outlet gas stream line, a CO_2 probe (Vaisala GMT 221, Finland) was fitted downstream from the glass exhaust water cooler to enable on-line measurement of CO_2 concentration. The bioreactor was heated at 80°C with water circulated into the double jacket using a heat bath equipped with a pump (Julabo SE 6, France). Bioreactor liquid volume and NaOH consumption, used to regulate culture pH, were monitored by a Combics 1 balance and a BP 4100 balance (both Sartorius, France), respectively. Temperature, pH, gas flow rates and stirrer speed were regulated through control units (local loops). All this equipment was connected to a Wago (France) automat via either a serial link (RS232/RS485), a 4–20 mA analog loop, or a digital signal. The automat was connected to a computer for process monitoring and data capture. BatchPro software (Decobecq Automatismes, France) was used to monitor and manage the process with good flexibility and accuracy.

Experimental conditions

In penicillin bottles, the best yield on O_2 for *S. solfataricus* cultivated on glucose was observed between 1.5 and 15% O_2 in air, although growth was optimal within the 1.5–24% range (Simon et al. 2009). As use of oxygen is paramount in phenol biodegradation, the experiments described in this study were run at 10% O_2. Air flow rate was regulated to maintain a constant in-reactor DOC of 1.5 mg l^{-1}, corresponding to 10% of O_2 in gas phase at 80°C. Total aeration was completed to 100 ml min^{-1} with nitrogen. pH was kept adjusted to 3.2 using NaOH (0.5 mM). Temperature was 80°C, and agitation speed was 300 rpm. Inoculum was prepared in 500 ml Erlenmeyer-flask cultures grown on standard mineral medium added with glucose (2000 mg l^{-1}) and incubated at 80°C and 200 rpm on a rotary shaker. The initial optical density of the inocula used was in the range 0.15–0.20.

To validate the cooling system device, an abiotic experiment with phenol (300 mg l^{-1}) was run over 4 days under the standard conditions given above. There was no loss in phenol or in water, as phenol concentration in the medium and reactor volume remained constant within this period. Samples were withdrawn manually or automatically every 4 h for glucose, phenol and growth measurements.

Analytical methods

Cell density was determined by measuring the optical density (OD) of the sample, after adequate dilution to keep OD measurements below 0.6, at 600 nm by UV spectrophotometry (WPA Biowave, France). Then, OD was converted to dry cell weight (X, mg l^{-1}) at the ratio 1000 mg l^{-1} biomass = 3.12 OD units. This value is very close to the 3.0 OD units per g of dried cells previously reported for *Sulfolobus solfataricus* ATCC 49155 (Nicolaus et al. 1991). For glucose and phenol measures, samples were centrifuged for 5 min at 14,000 rpm with an Eppendorf MiniSpin microcentrifuge.

Glucose was determined by HPLC using a differential refractometer detector (Shimadzu RID 6 A, Japan) connected to a computer running WINILAB III software (Perichrom, France). Twenty microliter of sample were loaded with a Spectra System AS 3000 autosampler onto an Aminex HPX-87H column (Biorad) set at 35°C and eluted at 0.5 ml min^{-1} with H_2SO_4 solution (0.75 mM). All analyses were repeated in triplicate.

Phenol analysis was performed by HPLC with a Waters apparatus composed of a 1525 binary pump, a 2996 UV/Vis diode array detector (λ_{max} for phenol = 270 nm), a Rheodyne injector (model 7725i) fitted with a 20-µl loop, a temperature control system and a degasser. Separation was carried out on a Symmetry C_{18} reversed-phase column (4.6 × 100 mm, ODS2, 5 µm) from Waters and protected with a guard cartridge. Elutions were performed at 30°C, at a flow rate of 0.75 ml min^{-1}, using a linear gradient of acetonitrile (A) in water acidified with 1% (v/v) acetic acid (B), in two steps: first, from 5 to 45% A for 25 min, and then from 45 to 100% A for 3 min (total 30 min). In these conditions, phenol had a retention time of 11.50 min. Data are averages of three measures. Volumetric phenol degradation rate (V_{phenol}, mg l^{-1} h^{-1}) was calculated from the slope of the linear portion of the phenol concentration vs. time plot.

Kinetics and respirometric parameters, mass balance

Specific growth rate (μ_X, h^{-1}) and specific phenol degradation rate (q_P, mg g^{-1} h^{-1}) were calculated from experimental data on biomass production and phenol consumption.

Oxygen consumption was estimated via the oxygen mass flow needed to maintain a constant DOC in the fermentation broth. It was assumed that oxygen consumption was only a function of metabolic activity. The oxygen yield factor $\left(Y_{X/O_2}, \text{g mol}^{-1}\right)$ was calculated from experimental data on oxygen fed to the reactor.

The experimental K_La was determined without biomass and according to the dynamic adsorption/desorption method described previously by Casas Lopez et al. (2006). Under the standard conditions given above, this value was 82.8 h^{-1}.

Carbon dioxide production rate (CDPR) was determined with the following equation:

$$\text{CDPR} = CO_2 \times F \left(\text{ml min}^{-1}\right) \quad (1)$$

where CO_2 is the carbon dioxide concentration in the exit gas (% v/v) and F is the total rate of flow fed into the reactor, F = 100 ml min^{-1}.

Total CO_2 production was calculated by integrating the CO_2 concentration values (% v/v) measured in the reactor exit gas throughout the experiments. At the pH used (3.2), it was assumed that the CO_2 dissolved in the medium was insignificant with respect to total CO_2 produced.

Biomass can be described by the empirical formula $CH_{1.8}O_{0.5}N_{0.2}$, giving a molecular weight of 24.6 g mol^{-1} (Feitkenhauer et al. 2001). Thus, the stoichiometric equation for phenol oxidation with biomass formation is:

$$C_6H_6O + Y_{O_2/P}O_2 + aNH_3 \rightarrow Y_{X/P}CH_{1.8}O_{0.5}N_{0.2} + Y_{CO_2/P}CO_2 + bH_2O \quad (2)$$

where $Y_{O_2/P}$, $Y_{X/P}$, $Y_{CO_2/P}$ are the molar phenol oxidation, biomass, and carbon dioxide yields, respectively. These yields were calculated from experimental data.

Thus, balancing Eq. 2 gives:

$$a = 0.2Y_{X/P}$$

$$b = 3 - 0.6Y_{X/P}$$

$$Y_{O_2/P} = 1 - 0.1\,Y_{X/P} + Y_{CO_2/P} \quad (3)$$

Hence, the $Y_{O_2/P}$ yield can be determined via two independent ways: from experimental data on oxygen and phenol consumption $\left(Y_{O_2/P_{exp}}\right)$ and from stoichiometric coefficients of phenol consumption $\left(Y_{O_2/P_{cal}}\right)$(Eq. 2) introducing experimental biomass and CO_2 yields on phenol (Eq. 3). These two ways are compared in the discussion section. Finally, a respiratory quotient was calculated, as defined below:

$$Q_{resp} = \frac{\Delta CO_2 prod}{\Delta O_2 cons} = \frac{Y_{CO_2/P}}{Y_{O_2/P}} \left(mol\ mol^{-1}\right) \quad (4)$$

Results

Adaptation of *S. solfataricus* to phenol

Phase 1: Growth on glucose and phenol

First, batch culture was inoculated with glucose-grown cells in a medium containing both glucose (2000 mg l^{-1}) and phenol (94 mg l^{-1}) as carbon and energy sources. After a 15-h lag-phase, biomass grew exponentially, reaching 700 mg l^{-1} after 41 h (Fig. 1). CDPR and growth curves showed similar

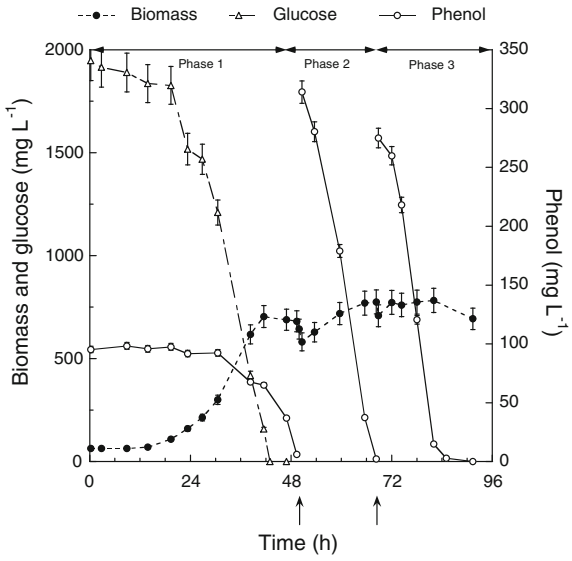

Fig. 1 Cell growth, glucose and phenol uptake for phenol biodegradation by glucose-grown cells of *S. solfataricus* 98/2. Initial phenol concentration: 94 mg l^{-1}. *Arrows* indicate phenol additions. All data are averages of three determinations. *Error bars* indicate STDEVs

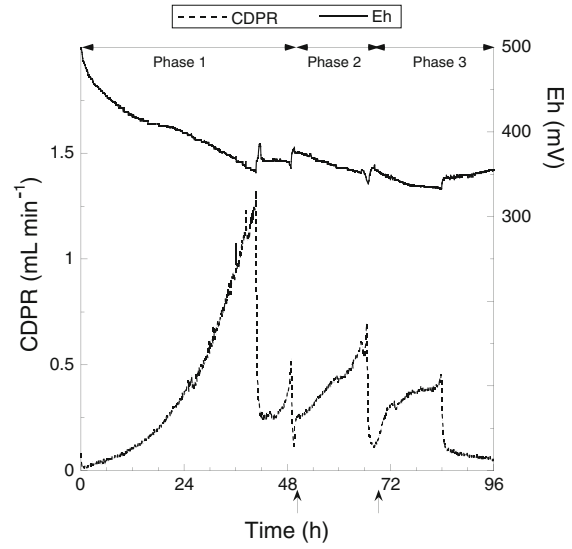

Fig. 2 Carbon dioxide production rate and culture redox potential evolution for phenol biodegradation by glucose-grown cells of *S. solfataricus* 98/2. Initial phenol concentration: 94 mg l^{-1}. *Arrows* indicate phenol additions

patterns (Fig. 2). In addition, the culture medium was reduced, as established by the drop in redox potential (E_h) from 515 to 345 mV (Fig. 2). After 30 h where only glucose was consumed, phenol uptake began while glucose was still present in the medium. When

glucose was exhausted, phenol consumption accelerated, with the remaining phenol (roughly 70%) being consumed within 7 h (Fig. 1), after which growth stopped and CDPR dropped drastically down to a constant value of 0.25 ml min^{-1} (therefore corresponding solely to phenol oxidation). Redox potential displayed a narrow peak (up to 380 mV), indicating glucose exhaustion, followed by a plateau at 365 mV. Phenol exhaustion translated as a new drop in CDPR and a new increase in E_h (Fig. 2).

Phases 2 and 3: Growth on phenol alone

After phase 1, phenol was added to the medium (314 mg l^{-1}) as sole energy and carbon source (phase 2). First, biomass promptly diminished to 630 mg l^{-1}, and a slow resumption in growth was observed as phenol was consumed (Fig. 1). An intense respiration took place, as illustrated by a high oxygen demand, since oxygen flow rate increased from 12.4 to 20 ml min^{-1} (data not shown). During the second phenol addition (275 mg l^{-1}, phase 3), no significant growth was observed, and phenol was merely oxidized to CO_2. This could indicate energy uncoupling, an inhibitory effect, or a lack in some nutrient. Volumetric phenol degradation rate (V_{phenol}) increased from 7.1 (phase 1) to 24.9 mg l^{-1} h^{-1} (phase 3). These rates are higher than for *S. solfataricus* P2 in flask cultures (5 mg l^{-1} h^{-1}) (Izzo et al. 2005), probably due to a higher biomass density (OD_{600} = 2.4 in this study vs. 0.6 in the P2 study (Izzo et al. 2005)). Each phenol addition triggered an immediate resumption in CO_2 production and a decrease in E_h (Fig. 2). Both CDPR and E_h were shown to be reliable indicators of substrate uptake and exhaustion. Although CDPR has a long history of use for following growth and substrate consumption, there is only one report dealing with the use of E_h for phenol monitoring (Santos and Rao 1996).

Biodegradation of phenol by phenol-grown cells of *S. solfataricus*

After two batch cultures on phenol (phenol concentration up to 350 mg l^{-1}), this experiment was started up by inoculating 1.63 l of fresh medium with 0.17 l of phenol-grown cells (10% v/v), and then feeding phenol into the reactor (324 mg l^{-1}). Results are presented in Fig. 3.

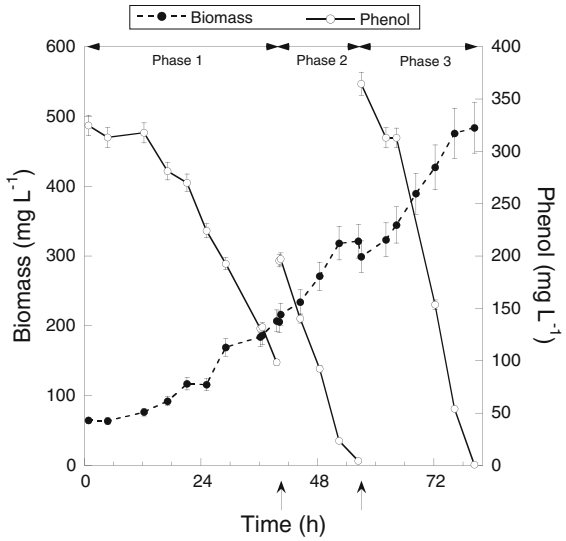

Fig. 3 Cell growth and phenol uptake for phenol biodegradation by phenol-grown cells of *S. solfataricus* 98/2. Initial phenol concentration: 325 mg l^{-1}. *Arrows* indicate phenol additions. All data are averages of three determinations. *Error bars* indicate STDEVs

After a short lag phase (<12 h), biomass grew exponentially up to 460 mg l^{-1} for a total phenol uptake of 1121 mg (in three additions) within 80 h. The three phases presented similar growth rates, and V_{phenol} increased with biomass from 8.05 to 16.6 mg l^{-1} h^{-1} in the third phase.

CDPR and oxygen flow rate patterns were similar to those observed as in the previous experiment (data not shown). Both parameters increased until phenol exhaustion, characterized by drops in both CDPR and oxygen demand. When phenol was re-fed into the reactor (365 mg l^{-1}), CDPR and subsequent oxygen flow rate again increased. Redox potential globally followed the same pattern as in the previous experiment (data not shown). When phenol was re-fed (4th addition), there was neither consumption nor respiration, and a dark brown pigment appeared in the medium, suggesting an accumulation of catechol, the hydroxylation product of phenol, in the culture, as previously reported for P2-strain phenol cultures (Izzo et al. 2005).

Discussion

After a series of tests, the difficulties and limitations commonly reported for operating high temperature

bioreactors (Nicolaus et al. 1991; Park and Lee 1997; Nemati et al. 2000; Worthington et al. 2003) were successfully overcome. The regulation devices made it possible to hold temperature, pH and DOC at their setpoints. The probes and the data capture system for CO_2, oxygen flow rate, redox potential, temperature and pH produced reliable data in this extreme environment.

Cultures often need to be acclimatized to phenol, and this can be achieved through enrichment cultivation methods (Contreras et al. 2008, Shourian et al. 2009) or repeated batches (Masqué et al. 1987). Conversely to previous conclusions (Chae et al. 2007), we demonstrated that *S. solfataricus* 98/2 was able to utilize phenol as carbon and energy sources. However, the adaptation was successful because the strain was grown on glucose with a small amount of phenol (94 mg l^{-1}). Indeed, we observed that when the cells were first grown on glucose alone and phenol was added at glucose exhaustion, the strain was unable to use phenol (data not shown). This ability to grow on phenol is consistent with the finding, in strain 98/2 genome, of a catechol 2,3-oxygenase (C23O) located within a putative multicomponent monooxygenase (MM) gene cluster matching exactly with the homologous region of the *S. solfataricus* (strain P2) genome (Chae et al. 2007). However, an insertion sequence (IS) exhibiting typical features of mobile elements has been identified in the putative MM protein A N-terminal fragment gene in strain 98/2, which could explain its apparent inability to grow on phenol (Chae et al. 2007). The ability of these mobile elements to move around the genome could explain the restoral of phenol degradation after adaptation. The adaptation to phenol involved the following successive steps:

1) First, glucose was consumed alone. Then, phenol uptake began even though glucose was still present in the medium, probably because at least one of the enzymes involved in phenol degradation was induced during the first 30 h. Unlike phenol-acclimatized activated sludge, where phenol consumption only began after total depletion of glucose (Bajaj et al. 2008), *S. solfataricus* 98/2 was able to activate both its glycolytic and phenol-degrading enzymatic machineries.
2) After glucose depletion, there was a respirometric period during which *S. solfataricus* 98/2 was only able to oxidize phenol alone without growth. This phase was characterized by a "stand-by" period lasting about 7 h, during which CDPR and E_h remained constant. When phenol uptake accelerated, respirometric activity resumed (end of phase 1).
3) A growth period where *S. solfataricus* was able to use phenol for biomass build-up (phase 2).

Table 3 Experimental kinetic and respirometric rates and yields of phenol biodegradation by glucose-grown and phenol-grown cells of *S. solfataricus* 98/2

Parameters	Glucose-grown cells			Phenol-grown cells		
	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
Substrate, concentration (mg l^{-1})	Glucose, 2000 + Phenol, 94	Phenol, 314	Phenol, 275	Phenol, 324	Phenol, 195	Phenol, 365
μ_X (h^{-1})	0.086[a]	0.021	0	0.035	0.033	0.027
q_P (mg g^{-1} h^{-1})	9.4	25.8	22.2	59.5	55.4	44.1
$Y_{X/P}$ (g mol^{-1})	nc	25.0	0	53.3	51.1	44.3
$Y_{CO_2/P}$ (mol mol^{-1})	nc	4.54	5.69	4.00	4.42	4.40
$Y_{O_2/P_{exp}}$ (mol mol^{-1})[b]	3.88[a]	5.83	6.10	5.67	5.66	5.78
$Y_{O_2/P_{cal}}$ (mol mol^{-1})[c]	nc	5.44	6.69	4.78	5.21	5.22
$Q_{resp\ exp}$ (mol mol^{-1})	0.70	0.78	0.81	0.70	0.78	0.76
$Q_{resp\ cal}$ (mol mol^{-1})	nc	0.83	0.85	0.84	0.85	0.84
$\left(Y_{X/O_2}\right)$ (g mol^{-1})	14[a]	4.3	0	9.4	9.0	7.6
Carbon recovery (%)	83	93	94	103	108	104

[a] On both glucose and phenol, [b] experimental values (from raw data), [c] values calculated from Eq. 3, *nc* not calculated

This study evidenced adaptation based on the fact that specific growth and specific phenol degradation rates (μ_X, q_P) were higher for phenol-grown cells (adapted cells) than glucose-grown cells (Table 3). Indeed, after several batches on phenol, μ_X increased from 0.021 to 0.034 h^{-1} and q_P increased from 9.4 to 59.5 mg g^{-1} h^{-1}, indicating a more rapid and efficient use of phenol for biomass build-up. This could be expected, since one of the enzymes putatively involved in the degradation is induced by phenol, and its activity increases with time (Ali et al. 1998). Both rates were constant during phase 1 and 2 for adapted cells but decreased in phase 3, which could indicate a limitation in certain nutrients. These values are markedly lower than those reported in the literature for mesophiles, such as $\mu_X = 0.38$ $h^{-1}$and $q_P = 390$ mg g^{-1} h^{-1} for *Candida* strain (Adav et al. 2007) and $\mu_X = 0.42$ h^{-1} for a *Pseudomonas putida* strain (Onysko et al. 2000). Values as high as $\mu_X = 2$ $h^{-1}$and $q_P = 1041$ mg g^{-1} h^{-1} were even reported for the thermophilic *Bacillus thermoleovorans* (Feitkenhauer et al. 2001). However, *Sulfolobus* species are generally characterized by low growth rates and only average conversion yields, whatever the strain or substrate (Table 2). The growth rates found in this study were higher than for strain P2 grown in Erlenmeyer flasks (0.021 h^{-1}, Izzo et al. 2005), probably due to a poorer oxygen transfer and lack of pH regulation in that study.

As expected, experimental $Y_{X/P}$ was similar between the 98/2 and P2 strains, whatever the culture conditions, i.e., 52.2 ± 1.1 g mol^{-1} in this study vs. 54 g mol^{-1} calculated from the data of Izzo et al. (2005). Nevertheless, the lower values for biomass yield on phenol and oxygen in phase 2 for glucose-grown cells ($Y_{X/P} = 25.0$ g mol^{-1}, $Y_{X/O_2} = 4.3$ g mol^{-1}) compared to phenol-grown cells (phase 1 and 2) could suggest a limitation in certain nutrients for biomass build-up or a partial adaptation of cells to phenol (Table 3). However, the best yields obtained in this study were lower than the yields reported for mesophiles, i.e., $Y_{X/S} = 84.6$ g mol^{-1} (*P. putida*, Onysko et al. 2000) or thermophiles, i.e., $Y_{X/S} = 94$ g mol^{-1} (*B. thermoleovorans*, Feitkenhauer et al. 2001).

Phenol has commonly been reported as a growth- and conversion yield-limiting substrate (Onysko et al. 2000; Khleifat 2006; Bai et al. 2007; Adav et al. 2007). This characteristic was reproduced here, as shown by the data on glucose + phenol (Phase 1 of glucose-grown cells) and the data reported in the same culture conditions on glucose (2000 mg l^{-1}). Indeed, the μ_X, $Y_{X/S}$ and (Y_{X/O_2}) obtained on glucose + phenol (0.086 h^{-1}, 54.5 g mol^{-1} and 14.0 g mol^{-1}, respectively) were slightly lower than those obtained on glucose alone (0.096 h^{-1}, 70.2 g mol^{-1} and 17.6 g mol^{-1}, respectively) (Cravero, personal communication), inhibitory effect of phenol on growth on glucose was also reported for the P2 strain, since P2-strain μ_X was roughly halved when phenol (1000 mg l^{-1}) was added to the medium (Chong et al. 2007). Given that at high phenol concentrations, inhibition effects are reportedly stronger (Onysko et al. 2000; Bai et al. 2007; Adav et al. 2007) and the energy spent to maintain cell membrane integrity is expectedly higher, yields and kinetic parameters would probably have been greatly improved by feeding phenol to maintain a lower concentration within the bioreactor (e.g., 50 mg l^{-1}) (Onysko et al. 2000).

Based on the data on phenol uptakes and biomass and CO_2 productions, together with a typical cellular composition of $CH_{1.8}O_{0.5}N_{0.2}$, we calculated a C-balance for each phase. For phenol-grown cells, C-balances were roughly similar for the 3 phases. Twice as much carbon was utilized for CO_2 (66–73%) than for biomass (31–37%). For glucose-grown cells, a much higher proportion of carbon from phenol was utilized for CO_2 production (76% for phase 2, and 94% for phase 3), and hence the proportion dedicated to biomass was much lower (17 and 0%, respectively). Considering biomass and CO_2 only, carbon recovery was around 100%, confirming that no other phenol metabolites accumulated during these runs (Table 3).

Despite the very low solubility of O_2 in water at 80°C, the highly effective oxygen feeding device and efficient regulation meant there was no O_2 limitation, thus allowing total mineralization of the phenol. By introducing $Y_{X/P}$ and $Y_{CO_2/P}$ in Eq. 3, $Y_{O_2/P}$ and the subsequent Q_{resp} from Eq. 4 were calculated and compared against values obtained from direct measurements of oxygen throughout the runs (Table 3). Except for phase 3 run with glucose-grown cells, the $Y_{O_2/P_{exp}}$ values remained roughly constant, whatever the state of the culture (growth or maintenance), at around 5.7 ± 0.2 mol mol^{-1}, which is consistent with values found in the literature, i.e., 5.8 mol mol^{-1} (Nikakhtari and Hill 2005; Feitkenhauer et al.

2003) but higher than the calculated values (Tables 1 and 3). The subsequent respiratory quotient ($Q_{respcal}$) calculated with $Y_{O_2/P_{cal}}$ is higher (between 0.82 and 0.87 mol mol^{-1}) and closer to the theoretical value (0.87 mol mol^{-1}) than $Y_{O_2/P_{exp}}$ (between 0.70 and 0.81 mol mol^{-1}). This difference could be explained by a possible error inherent to the K_La determination, and a subsequent underestimation of $Y_{O_2/P_{exp}}$. It can thus be concluded that experimental carbon data were more reliable than oxygen data.

Finally, the degradation of phenol by *S. solfataricus* 98/2 indirectly proves the activity of a catechol dioxygenase, which is the key enzyme opening the aromatic ring. This in turn would suggest its potential to metabolize a large number of aromatic hydrocarbons such as cresols, benzene, toluene, and even polycyclic aromatic hydrocarbons (Sei et al. 1999; Duarte da Cunha et al. 2006). Moreover, as no additional C source was needed, this thermoacidophilic microorganism appears an excellent candidate for field applications in harsh environments.

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