Revisit to the biodesulfurization capability of hyperthermophilic
archeon Sulfolobus Solfataricus P2 revealed DBT consumption
by the organism in an oil/water two-phase liquid system at high
temperatures

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Running head: Biodesulfurization capability of S. solfataricus
Abstract

The ability of hyperthermophilic archaeon, *Sulfolobus solfataricus* P2, to grow on organic and inorganic sulfur sources was investigated. A sulfur free mineral medium has been employed with different sources of carbon. Results showed that inorganic sulfur sources display growth curve patterns significantly different from the curves obtained with organic sulfur sources. *Solfataricus* has an ability to utilize DBT and its derivatives, but it lacks BT utilization. *Solfataricus* utilizes DBT at a rate of 1.23 µmol 2-HBP h\(^{-1}\) g DCW\(^{-1}\) even at 78 °C, at which DBT is known to be unstable. After enabling DBT stabilization using a two-phase culture system, stable microbial growth was achieved showing a desulfurization rate of 0.34 µM DBT g DCW\(^{-1}\) h\(^{-1}\). *Solfataricus* offers beneficial properties compared to the other desulfurizing mesophilic/moderate thermophilic bacteria due to its capacity to utilize DBT and its derivatives under hyperthermophilic conditions.

**Keywords:** Biodesulfurization, dibenzo thiophene; gas chromatography; *Sulfolobus solfataricus* P2; sulfur compounds
1. Introduction

Combustion of fossil fuels leads to the atmospheric emission of sulfur oxides that contribute to acid rain and air pollution.\(^1\) Strict government regulations throughout the world have been implemented to reduce these emissions.\(^2\) Nowadays, the current technology used to reduce the sulfur composition in fuels is hydrodesulfurization (HDS), which is the conventional method carried out with chemical catalysis at high temperature (290-450 °C) and pressure (1-20 mPa).\(^1\) Heterocyclic organosulfur compounds [dibenzothiophene (DBT) and substituted DBTs] represent the significant sulfur (up to 70%) quantities in petroleum and are recalcitrant to HDS.\(^3\) Therefore, biological desulfurization (BDS) using microorganisms and/or enzymes are an attractive alternative or complementary method to HDS due to its low cost, mild reaction conditions and greater reaction specificity.\(^4\)

DBT is a widely used model compound in research for desulfurization studies.\(^5\) Sulfur-specific cleavage of DBT (4S pathway) is a preferable pathway in biodesulfurization, in which DBT is selectively removed without carbon skeleton rupture. This pathway includes four reactions through the conversion of DBT into a free sulfur product, 2-hydroxybiphenyl (HBP) and sulfite/sulfate.\(^6\)

Various DBT desulfurizing microorganisms have been reported to date; for instance, mesophilic bacteria such as *Rhodococcus* sp. IGTS8,\(^7\) *Rhodococcus erythropolis* H-2,\(^8\) *Corynebacterium* sp.,\(^9\) *Bacillus subtilis* WU-S2B\(^10\) and a moderately thermophilic *Mycobacterium phlei* WU-F1\(^11\) are known to use the 4S pathway in DBT desulfurization. Since these bacteria exhibit high DBT-desulfurization ability at around 30 °C and 50 °C for mesophilic and moderately thermophilic bacteria, respectively; their usage in fossil fuel desulfurization as an alternative or...
complementary to hydrodesulfurization requires an additional cooling process of the
fuel to ambient temperature following HDS. This additional cooling process causes
an economical burden when used for large scale fossil fuel desulfurization. Thus,
hyperthermophilic microbial desulfurization is desirable and makes the crude oil
biodesulfurization process more feasible due to low viscosity of the crude at high
temperature.  

There were various attempts to use hyperthermophiles in biodesulfurization to date, 12-
15 yet most of these studies were able to clearly delineate the pyritic sulfur
desulfurization, but lack to show reliable sufficient amounts of organic sulfur removal
efficiency. A study that undertook the usage of a hyperthermophilic *Sulfolobus*
*acidocaldarius* in DBT utilization revealed the oxidation of sulfur present in DBT to
sulfate at 70 °C. 13 Unfortunately, that study did not include DBT degradation at high
temperatures in the absence of microorganism 13, therefore the obtained rate of
desulfurization could not represent the real biodesulfurization rate. Another attempt to
study heterocyclic organosulfur desulfurization using a thermophile, *Sulfolobus*
solfataricus DSM 1616 15 at 68 °C showed DBT self-degradation in the absence of
microorganism at high temperatures, thus no substantial DBT utilization could be
observed. This study clearly pointed the difficulty to use DBT model compound at
high temperatures in biodesulfurization by *S. solfataricus*. 15 Nonetheless, the same
study showed the oxidation of thiophene-2-carboxylate by *S. solfataricus*, 15 therefore
organic sulfur desulfurization molecular mechanism had shown to be present in this
hyperthermophile, and further investigations are necessary to optimize the conditions
for better organic sulfur removal with possibly a different *Sulfolobus* strain, which
might lead to better efficiency for desulfurization.

Hyperthermophiles are isolated mainly from water containing volcanic areas such as
solfataric fields and hot springs in which they are unable to grow below 60 °C. *Sulfolobus solfataricus* P2 belonging to archaeabacteria grows optimally at temperatures between 75 and 85 °C and at low pHs between 2 and 4, utilizing a wide range of carbon and energy sources.

This paper describes the potential of a hyperthermophilic archaean, *S. solfataricus* P2, to utilize several inorganic and organic sources of sulfur for growth in various conditions, and shows *S. solfataricus* P2’s ability to remove sulfur from DBT via the sulfur-selective pathway even under high temperatures with the elimination of DBT self-degradation. To the best of our knowledge, this is the first report showing the DBT desulfurization kinetics analysis of *S. solfataricus* P2.

2. Results and discussion

2.1 Carbon source influence on the growth of *S. solfataricus* P2

The ability of *S. solfataricus* P2 to use several sources of carbon was investigated. Four types of carbon sources have been applied to the SFM medium: D-glucose, D-arabinose, D-mannitol (Figure 1) and ethanol. All these experiments have been carried out employing 2 g l⁻¹ as the initial concentration of carbon source. Figure 2 shows the effects of different sources of carbon on archaenal growth. The highest growth rate, 0.0164 h⁻¹(60.9 h), and the maximum biomass density, 0.149 g dry weight l⁻¹, were observed when D-glucose was employed as a carbon source (Figure 2). On the other hand, D-arabinose, D-mannitol and ethanol (at a concentration of 2 g l⁻¹) did not support the growth (Figure 2). Our data in Figure 2 clearly showed that glucose is a better carbon source for the growth of *S. solfataricus* P2 compared to the tested other carbon sources. *S. solfataricus* harbors a semi-phosphorylative Entner-Doudoroff (ED) pathway for sugar metabolism.¹⁹, ²⁰
Since D-glucose is the first metabolite necessary to initiate glycolysis, it is rather expected to observe better D-glucose utilization than the other sugars. For both D- and L-arabinose a well-defined pentose mechanism exists in 	extit{S. solfataricus}. Both pentose mechanisms may include intermediates that are not heat stable, thus these products may get degraded while enough ATP gets accumulated to allow cells to survive. As presented with a recent study, unstable intermediate metabolites exist for semi-phosphorylative ED pathway in glucose metabolism for hyperthermophiles that grow at extreme temperatures, therefore similar type of unstable intermediate production in the pentose mechanism may prevent the growth of 	extit{S. solfataricus} cells under scarce sugar supplies.

To further determine the optimum growth condition of 	extit{S. solfataricus} P2 in SFM medium when glucose is the source of carbon, various concentrations of glucose ranging from 2 g l$^{-1}$ to 20 g l$^{-1}$ on SFM culture were employed. The results revealed that the highest growth rate; 0.0339 h$^{-1}$ (29.5 h) and biomass concentration; 0.157 g l$^{-1}$ were obtained when 20 g l$^{-1}$ of glucose was used (Figure 3). It can be affirmed that the higher the glucose concentration, the higher the growth rate is (Table 1). Figure 3 also indicates that with increasing concentrations of glucose, enhanced growth rate was observed, and the time required to reach the maximum biomass value was decreased; however the maximum cell densities obtained with increasing concentrations of glucose were similar for all of the concentrations (ranging from 0.14 to 0.157 g DCW l$^{-1}$). At the same time, the lag time decreased with the highest concentration of glucose application, and cells reached to the stationary phase faster as the concentration of glucose was increased. One explanation for the observed increased rate for the growth with higher glucose concentration might due to allowing cells steadily obtain all the necessary intermediate metabolites,
even some of them get degraded under high temperatures,\textsuperscript{20} still excess amounts for productive glycolytic cycles would be enough for cells to proliferate. Although, an acceptable growth profile was observed when glucose was employed as the carbon source; overall, in SFM medium, presence of glucose was not sufficient to obtain an optimal growth, additional micronutrients were necessary to optimize the growth conditions.

2.2 Organic sulfur compounds utilization

The ability of \textit{S. solfataricus} P2 to utilize organic sulfur compounds was evaluated toward 4,6-DMDBT, DBT sulfone, DBT and BT. Each was acted as a sole source of sulfur for the growth with an initial concentration of 0.3 mM in SFM culture except the presence of trace amounts of sulfur originating from the culture stocks. ICP-OES analysis revealed the presence of 0.00168 ± 0.0008 g l\textsuperscript{-1} sulfur in the 100 ml control flasks. Unless otherwise noted, all the cultivation experiments were done in the same manner, and their initial sulfur contents were estimated to be similar to the initially determined value. Also for all of the growths, 20 g l\textsuperscript{-1} of glucose was employed as a carbon source in SFM medium. The effects of the organic sulfur compounds on the growth are shown in Figure 4. When the cultures were incubated initially with DBT, DBT-sulfone, 4,6-DMDBT and BT, there were no archaeal growth (data not shown). Instead of employing organic compounds in the beginning of the growth, each organic sulfur compound was separately added into SFM medium after a moderate optical density (OD between 0.35 and 0.4, around the midst of log phase during \textit{S. solfataricus} P2 growth) was attained. Thus, supplementation of organic compounds in this way enabled \textit{S. solfataricus} P2 cells to grow well on media containing DBT-sulfone and 4,6-DMDBT as the sole sources of sulfur; but addition of BT resulted abrupt interruption of cell growth, and
subsequently led the cells to death (Figure 4). DBT addition, on the other hand, progressively ceased the growth of the cells (Figure 4). Maximum biomass densities and specific growth rates are given in Table 2. Maximum cell density was achieved with 4,6-DMDBT, yielding 2.5 times higher cell density compared to that of the control. DBT-sulfone presence enabled cells to achieve 1.4 times higher cell density with respect to the control. These results revealed that *S. solfataricus* P2 can utilize organic sulfur compounds containing DBT and its derivatives; but, even among them, it has certain preference to some types of organic molecules than the others. Results indicated that *S. solfataricus* P2 cannot utilize BT. Since DBT and BT desulfurization pathways were shown to be different for various desulfurizing bacteria,¹⁶, ²¹ it can be concluded that *S. solfataricus* P2 has a metabolic pathway specific for DBT and its derivatives.

2.3 Inorganic sulfur compounds utilization

To compare the effects of the organic and inorganic sulfur sources on growth, 0.3 mM inorganic sulfur sources as a sole sulfur source; elemental sulfur, sodium sulfite, sodium sulfate, potassium persulfate and potassium disulfite were employed into the SFM medium at OD₆₀₀ around 0.32. Growth curve patterns of cultures containing inorganic sulfur sources were similar except for the elemental sulfur case (Figure 5). All the growth curves reveal a short stationary period after supplementation of the inorganic sulfur compounds, suggesting a certain adaptation time for the cells to the new nutrient environment. This adaptation period may correlate to the immediate uptake of inorganic sulfur molecules by the cells. A logarithmic enhancement in the growth followed by this short stationary period shows that *S. solfataricus* P2 utilizes the supplied inorganic sulfur sources. Similar growth rates were observed for the sulfate and
sulfite present cases (Table 3). Elemental sulfur supplemented growth revealed a
longer adaptation period and showed a slower growth rate compared to that of the
sulfate and sulfite supplemented growths (Table 3). The growth curves showed
maximum cell densities with the sulfate compounds, a very similar maximum cell
density (0.651 g DCW l⁻¹) with minor errors were obtained (Table 3). Inorganic
sulfur sources led to a rapid cell death after a maximum biomass cell density was
obtained except for the elemental sulfur employed case, which showed a sustained
stationary phase (Figure 5) after a maximum cell density, 0.586±0.016 g DCW l⁻¹
was reached (Table 3). Rapid cell death after sulfate and sulfite utilization could be
explained by the excess uptake of these anions by the cells leading to a demand for
counter ion balance, which can be maintained by excess accumulation of cations to
cells causing an osmotic imbalance. The observation of prolonged stationary phase
in the elemental sulfur present case was similar to that of the control growth where
even after 150 h of growth in the stationary phase still a certain cell density can be
measured but the estimated cell density for the control was almost 4 times less than
the elemental sulfur supplemented trial (Figure 5, Tables 1 and 3). In SFM medium,
when inorganic sulfur sources were used as the sole sulfur source instead of
organic sulfur compounds, faster growth rates and biomass concentrations were
observed for *S. solfataricus* P2. It is thought that not all glucose was used after cells
reach to a cell density of 0.157 g DCW l⁻¹. At this point, sulfur became the growth
limiting factor and supplementation of inorganic sulfur sources led to faster growth
and higher biomass density.

2.4 DBT consumption kinetics by *S. solfataricus* P2

Our results revealed that *S. solfataricus* P2 can utilize 4,6-DMDBT and DBT
sulfone efficiently, but DBT utilization was not as effective as the former
compounds in SFM culture medium. Since DBT has been used as the model molecule of the thiophenic compounds present in fossil fuels, we aimed to optimize DBT utilization levels of *S. solfataricus* P2 by changing the growth medium conditions. Addition of yeast extract in the minimal medium significantly enhanced the utilization levels of DBT by *S. solfataricus* P2. The effect of different concentrations of DBT was tested in the growth of *S. solfataricus* P2 (Table 4); and with 0.1 mM DBT supplementation, cell density was enhanced significantly compared to the control, where no DBT was added in the minimal medium, and to the increasing DBT concentrations. Higher amounts of DBT usage showed significantly lower maximum cell density; and therefore 0.1 mM of DBT was used in our DBT desulfurization kinetics studies (Table 4). A continuous growth was observed until 89 h with a simultaneous production of 2-HBP, which was determined by both Gibbs assay and GC (Figure 6). It was observed that DBT concentration decreased sharply under abiotic conditions (data not shown). Earlier work also revealed DBT to be unstable at higher temperatures in aqueous environment. However, even under these conditions, desulfurization activity was observed in growing cultures, and is estimated to be 1.23 μmol 2-HBP h⁻¹ g DCW⁻¹. Specific production rate of 2-HBP was decreased sharply after 16.5 h as can be seen in Figure 7. Similar abrupt decrease in the production rate of 2-HBP was observed earlier in most of the BDS studies, and was explained by the production of HBP in the medium causes substrate inhibition type of enzyme kinetics (26). Although 93% of DBT depletion was observed within 39 h, 2-HBP production was continued to increase up until 114 h to a concentration of 47.6 μM. Growth of *S. solfataricus* P2 stopped near the maximum levels of 2-HBP was produced (Figure 6). Similar growth inhibition behavior by 2-HBP production was
also observed in previous BDS studies.\textsuperscript{27,28} It was reported that 2-HBP above 200 μmol/l was toxic to the bacterial cells and inhibitory to biodesulfurization.\textsuperscript{8} Even though, the maximum levels of produced 2-HBP concentration in our studies were not close to the toxic level, but still a decrease in 2-HBP production rate was observed with cell death. Another explanation may be the development of other products in the biodesulfurization pathway becoming toxic to cells.

Since DBT was not stable at 78 °C in aqueous environment (90% DBT depletion was observed within 16.5 h (data not shown)), an oil phase was used to prevent the effects of temperature and aqueous medium on DBT stabilization. DBT was preserved under abiotic conditions when the xylene was used as the second phase. Although addition of xylene containing DBT ceased the growth at the mid-log phase, 22% DBT utilization was observed within 72 h (Figure 8). The specific rate of DBT degradation in the first 23 h was 0.34 μM DBT g DCW$^{-1}$ h$^{-1}$. After 24 h of xylene addition, \textit{S. solfataricus} P2 secreted a biosurfactant into the culture medium. Emulsification was observed only in growing cultures not in the control. It was suggested in a previous study that formation of biosurfactant may have a role on the DBT desulfurization process by increasing the contact surface of cells with the oil phase.\textsuperscript{29} Two phase system has been studied in many BDS studies in which hexane, heptane and xylene were mainly used as the oil phase.\textsuperscript{29, 30} Since the growing temperature necessary for the \textit{S. solfataricus} P2 growth was relatively higher than other BDS studies used in the two phase systems,\textsuperscript{29-32} an oil having high boiling temperature, xylene (bp. 134-139 °C), was selected as the oil phase. Although DBT containing xylene phase ceased the growth of the microorganism when it is supplied in the two phase system at 40% (v/v), equilibrium between xylene concentration, amount of DBT in oil phase and initial cell concentration can be optimized for
effective DBT biodesulfurization when applied to industrial usage.

Two oil phase system has been used for enhancing the poor solubility of many organic compounds in aqueous cultures.\textsuperscript{31, 32} Since the solubility of DBT is 0.005 mM in water,\textsuperscript{32} aqueous/apolar culture system has an advantage on the biodesulfurization of DBT and its derivatives.

In conclusion, since biodesulfurization done under high temperatures offers a potential for an alternative/complementary method for lowering the sulfur content of fossil fuels, in that respect, hyperthermophilic \textit{S. solfataricus} P2 with its potential DBT-desulfurization ability can serve as a model system for the efficient biodesulfurization of fossil fuels. Further molecular biology studies for the characterization of the genes responsible for DBT desulfurization, undertaken already by our group, will enable to delineate the exact BDS mechanism of \textit{S. solfataricus} P2.

3. Experimental

3.1 Chemicals

\textit{S. solfataricus} was obtained as a powder from American Type Culture Collection (ATCC\textsuperscript{(R)} 35091\textsuperscript{TM}). DBT (99\%) was obtained from Acros Organics, DBT-sulfone (97\%) was from Sigma Aldrich, 4,6-Dimethyl dibenzothiophene (97\%), elemental sulfur (99\%), were from ABCR, DMF was from Riedel-de Haën. All other reagents were of the highest grade commercially available.

3.2 Culture media and growth conditions

Sulfur-free mineral (SFM) medium was prepared by dissolving 70 mg of CaCl\textsubscript{2}.2H\textsubscript{2}O, 1.3 g NH\textsubscript{4}Cl, 0.25 g MgCl\textsubscript{2}.6H\textsubscript{2}O, 0.28 g KH\textsubscript{2}PO\textsubscript{4} and 0.5 ml trace elements solution in 1 l of milli-Q water, and this mix was adjusted to pH 3 with HCl. Trace elements solution\textsuperscript{16} was prepared with 25 g l\textsuperscript{-1} EDTA, 2.14 g l\textsuperscript{-1} ZnCl\textsubscript{2}.\textsuperscript{29}
2.5 g l\(^{-1}\) MnCl\(_2\).4H\(_2\)O, 0.3 g l\(^{-1}\) CoCl\(_2\).6H\(_2\)O, 0.2 g l\(^{-1}\) CuCl\(_2\).2H\(_2\)O, 0.4 g l\(^{-1}\) NaMoO\(_4\).2H\(_2\)O, 4.5 g l\(^{-1}\) CaCl\(_2\).2H\(_2\)O, 2.9 g l\(^{-1}\) FeCl\(_3\).6H\(_2\)O, 1.0 g l\(^{-1}\) H\(_3\)BO\(_3\), 0.1 g l\(^{-1}\) KI. Minimal medium\(^{17}\) was adjusted to pH 3 and supplemented with yeast extract (0.15% w/v) and glucose (20 g l\(^{-1}\)). Initial stocks of \textit{S. solfatarius} culture were initially made by using minimal medium and kept at -80 °C as 10% glycerol stocks of 1 ml aliquots. Cell cultivation was carried out at 78 °C in 250 ml flasks containing 100 ml of medium with 160 rpm shaking.

3.3 Carbon utilization

SFM culture medium was employed as the base medium and was supplemented with D-arabinose, ethanol, D-glucose and D-mannitol as different sources of carbon to a final concentration of 2 g l\(^{-1}\). To find out the optimum sulfur free growth condition, various concentrations of the most effective carbon source, glucose, was added on SFM culture medium at concentrations of 2, 5, 10, 15 and 20 g l\(^{-1}\). Data are represented as the means of triplicate cultures ± standard error.

3.4 Sulfur utilization

The ability of \textit{Sulfolobus solfatarius} P2 to utilize organic and inorganic sulfur sources was investigated. Several organic and inorganic sulfur compounds including DBT, BT, DBT-sulfone, 4,6-dimethyldibenzothiophene (4,6-DMDBT), elemental sulfur, sodium sulfide, sodium sulfate, potassium persulfate and potassium disulfite were added with an initial concentration of 0.3 mM to SFM culture as the sole source of sulfur. However, there is a trace amount of sulfur contaminating from the stocks of the culture, which were first prepared using minimal medium. Sulfur content originating from the stocks of \textit{S. solfatarius} in SFM was measured using inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin-Elmer,
USA) as described in a previous study.\(^1\) In all of these media, 20 g l\(^{-1}\) of glucose was used as the sole source of carbon. SFM culture containing only the carbon source (20 g l\(^{-1}\) of glucose) was used as a control. Stock solutions of organic sulfur compounds, DBT, BT, 4,6-DMDBT and DBT-sulfone were dissolved in N,N-dimethylformamide (100 mM). In all of these experiments, organic sulfur compounds were added to the growth culture after a certain exponential growth was achieved, corresponding to an OD\(_{600}\) (optical density at 600 nm) value in between 0.35 and 0.4. Data are represented as the means of triplicate cultures ± standard error.

For desulfurization kinetics assay, minimal medium supplemented with 0.1 mM DBT, 0.15% w/v yeast extract and glucose (20 g l\(^{-1}\)) was used in the presence and absence of 40% (v/v) xylene. Cells grown at the mid-log phase (OD\(_{600}\) being 1.5) were supplemented with DBT or DBT dissolved in xylene in a two-state oil phase.

3.5 Analytical methods

Cell densities were measured at the 600 nm wavelength using a Shimadzu UV visible spectrophotometer (model UV-1601). A correlation between OD\(_{600}\) and dry cell weight (DCW) was done to determine the concentration of cells. One unit of optical density corresponded to 0.44 g DCW l\(^{-1}\).

3.6 Analysis of organic sulfur compounds and metabolites

For gas chromatography (GC) experiments, aliquots of the culture during the course of bacterial growth were acidified below pH 2.0 with 1 N HCl, then culture was extracted with equal volumes of ethyl acetate during a 5 min vortex and 10 min centrifugation at 2000 rpm. For the two-phase system, xylene fractions were directly used for DBT quantification. 2 µl of the organic fraction was used for the detection
of DBT and 2-HBP by using a GC (HP-Agilent Technologies 6890N GC Systems, USA) equipped with a flame ionization detector. Agilent JW Scientific DB-5 capillary 30.0 m × 0.25 mm × 0.25 µm column was used for the measurements. Temperature was set to 50 °C for 5 min followed by a 10 °C min⁻¹ rise up to 280 °C and kept at this temperature for 5 min. Injector and detector temperatures were both maintained at 280 °C. Quantification of DBT and 2-HBP were performed using standard curves with a series of dilutions of the pure DBT and 2-HBP compounds as a reference. All the reaction mixtures were prepared as triplicates.

3.7 Gibb’s assay / Desulfurization assay

The Gibb’s assay was used in conjunction with GC analyses to detect and quantify the conversion of DBT to 2-HBP produced by the Sulfolobus solfataricus P2 in the culture media lacking xylene. The assay was carried out as follows: 1 ml of culture was adjusted to pH 8.0 with 10% (w/v) Na₂CO₃, then 20 µl of freshly prepared Gibb’s reagent (2,6-dicholoroquinone-4-chloroimide, 5 mM in ethanol) was added. The reaction mixtures were allowed to incubate for 60 min at 30 °C for color development. The mixtures were then centrifuged at 5000 rpm for 10 min to remove cells, and absorbance of the supernatant was determined at 610 nm (UV 1601, Shimadzu, Japan). Concentration of produced 2-HBP from the Gibb’s assay results was determined from the standard curve obtained by different concentrations of pure 2-HBP. Results correspond to the means of three different experiments with the standard errors included.

Acknowledgements

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Table 1. Calculated growth rates and maximum cell densities corresponding to experimental growth data of *S. solfataricus* P2 cells when treated with increasing glucose concentrations as the sole source of carbon

<table>
<thead>
<tr>
<th>Glucose Concentration (g L⁻¹)</th>
<th>Growth Rate (h⁻¹)</th>
<th>Maximum Cell Density (g L⁻¹)</th>
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<tbody>
<tr>
<td>2 g.L⁻¹ glucose</td>
<td>0.0164 ± 0.0006</td>
<td>0.149 ± 0.008</td>
</tr>
<tr>
<td>5 g.L⁻¹ glucose</td>
<td>0.0192 ± 0.0004</td>
<td>0.148 ± 0.003</td>
</tr>
<tr>
<td>10 g.L⁻¹ glucose</td>
<td>0.0217 ± 0.0006</td>
<td>0.139 ± 0.002</td>
</tr>
<tr>
<td>15 g.L⁻¹ glucose</td>
<td>0.0276 ± 0.0014</td>
<td>0.149 ± 0.005</td>
</tr>
<tr>
<td>20 g.L⁻¹ glucose</td>
<td>0.0345 ± 0.0011</td>
<td>0.199 ± 0.003</td>
</tr>
</tbody>
</table>
Table 2. Various organic sulfur compound utilization by *S. solfatarius* P2 in SFM medium

<table>
<thead>
<tr>
<th></th>
<th>growth rate (h⁻¹)</th>
<th>maximum cell density (g l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6 DMDBT</td>
<td>0.0172 ± 0.0011</td>
<td>0.423 ± 0.031</td>
</tr>
<tr>
<td>DBT-sulfone</td>
<td>0.0179 ± 0.0056</td>
<td>0.281 ± 0.011</td>
</tr>
<tr>
<td>BT</td>
<td>-</td>
<td>0.192 ± 0.009</td>
</tr>
<tr>
<td>DBT</td>
<td>-</td>
<td>0.183 ± 0.004</td>
</tr>
</tbody>
</table>
Table 3. Various inorganic sulfur compound utilization by *S. solfataricus* P2 in SFM medium

<table>
<thead>
<tr>
<th></th>
<th>growth rate (h(^{-1}))</th>
<th>maximum cell density (g l(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elemental S</td>
<td>0.0165 ± 0.0012</td>
<td>0.586 ± 0.016</td>
</tr>
<tr>
<td>Sodium sulfite</td>
<td>0.0226 ± 0.0006</td>
<td>0.628 ± 0.053</td>
</tr>
<tr>
<td>Potassium disulfite</td>
<td>0.0254 ± 0.0005</td>
<td>0.623 ± 0.008</td>
</tr>
<tr>
<td>Sodium sulfate</td>
<td>0.0220 ± 0.0008</td>
<td>0.651 ± 0.005</td>
</tr>
<tr>
<td>Potassium persulfate</td>
<td>0.0222 ± 0.0003</td>
<td>0.651 ± 0.001</td>
</tr>
</tbody>
</table>
Table 4. Utilization of increasing DBT concentrations by *S. solfataricus* P2

<table>
<thead>
<tr>
<th>DBT Concentration</th>
<th>Growth Rate (h⁻¹)</th>
<th>Maximum Cell Density (g l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mM DBT</td>
<td>0.0122 ± 0.0014</td>
<td>2.19 ± 0.28</td>
</tr>
<tr>
<td>0.2 mM DBT</td>
<td>0.0061 ± 0.0011</td>
<td>2.13 ± 0.11</td>
</tr>
<tr>
<td>0.3 mM DBT</td>
<td>0.0020 ± 0.0002</td>
<td>0.87 ± 0.01</td>
</tr>
<tr>
<td>0.4 mM DBT</td>
<td>-</td>
<td>0.73 ± 0.01</td>
</tr>
<tr>
<td>Yeast Medium (Control)</td>
<td>0.0149 ± 0.0010</td>
<td>1.57 ± 0.05</td>
</tr>
</tbody>
</table>
Figure 1: Some of the carbon sources used in the study. Molecular structures of D-glucose, D-arabinose and D-mannitol are shown.
**Figure 2:** Effects of different carbon compounds (concentrations of 2 g l$^{-1}$) on the growth of *S. solfataricus* P2 in SFM medium. (o) D-mannitol, (●) D-arabinose, (+) ethanol, (★) D-glucose. The symbol star represents the highest growth rate observed for D-glucose.
Figure 3: Glucose gradients from 2 g l\(^{-1}\) to 20 g l\(^{-1}\) were performed in SFM medium. (●) 2, (○) 5, (▼) 10, (▽) 15 and (■) 20 g l\(^{-1}\) glucose
**Figure 4:** Growth of *S. solfataricus* P2 in the presence of 0.3 mM organic sulfur sources in SFM medium supplemented with 20 g l\(^{-1}\) glucose. (●) BT, (○) 4-6 Dimethyldibenzothiophene, (▼) DBTsulfone, (▽) DBT and (-) SFM-only medium. Sulfur sources were supplemented to the growing cultures at OD\(_{600}\) near 0.4
**Figure 5:** Growth of *S. solfataricus* P2 in the presence of 0.3 mM inorganic sulfur sources in SFM supplemented with 20 g l⁻¹ glucose. (▼) elemental sulfur, (○) sodium sulfite, (■) sodium sulfate, (▽) potassium persulfate, (●) potassium disulfite and (□) SFM-only medium. Sulfur sources were supplemented to the growing cultures at OD600 near 0.4
Figure 6: Formation of 2-HBP by the growing cells of *Sulfolobus solfataricus* P2. DBT was supplemented to growing cultures in minimal medium at 0.66 g dry cell l$^{-1}$. (▲) DCW, (●) 2-HBP
Figure 7: The time course of specific production rate of 2-HBP from 0.1 mM DBT by *Sulfolobus solfataricus* P2
Figure 8: Consumption of DBT. Experiments were performed in minimal medium containing 40% (v/v) xylene