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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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 ^{o}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, dibenzothiophene; 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 pheli* 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.[3]

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 ^{o}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 ^{o}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 archaebacteria 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 archaeon, *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 archaeal 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-phosporylative 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 *S. solfataricus*.[19] 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-phosporylative ED pathway in glucose metabolism for hyperthermophiles that grow at extreme temperatures,[20] therefore similar type of unstable intermediate production in the pentose mechanism may prevent the growth of *S. solfataricus* cells under scarce sugar supplies.

To further determine the optimum growth condition of *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,[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 *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^{-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^{-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 *S. solfataricus* P2 growth) was attained. Thus, supplementation of organic compounds in this way enabled *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 OD_{600} 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.[15] 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,[22-25] 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

249 also observed in previous BDS studies.[27, 28] It was reported that 2-HBP above 200
250 μmol/l was toxic to the bacterial cells and inhibitory to biodesulfurization.[8] Even
251 though, the maximum levels of produced 2-HBP concentration in our studies were
252 not close to the toxic level, but still a decrease in 2-HBP production rate was
253 observed with cell death. Another explanation may be the development of other
254 products in the biodesulfurization pathway becoming toxic to cells.
255 Since DBT was not stable at 78 ^{o}C in aqueous environment (90% DBT depletion
256 was observed within 16.5 h (data not shown)), an oil phase was used to prevent the
257 effects of temperature and aqueous medium on DBT stabilization. DBT was
258 preserved under abiotic conditions when the xylene was used as the second phase.
259 Although addition of xylene containing DBT ceased the growth at the mid-log phase,
260 22% DBT utilization was observed within 72 h (Figure 8). The specific rate of DBT
261 degradation in the first 23 h was 0.34 μM DBT g DCW^{-1} h^{-1}. After 24 h of xylene
262 addition, S. *solfataricus* P2 secreted a biosurfactant into the culture medium.
263 Emulsification was observed only in growing cultures not in the control. It was
264 suggested in a previous study that formation of biosurfactant may have a role on the
265 DBT desulfurization process by increasing the contact surface of cells with the oil
266 phase.[29] Two phase system has been studied in many BDS studies in which hexane,
267 heptane and xylene were mainly used as the oil phase.[29, 30] Since the growing
268 temperature necessary for the *S. solfataricus* P2 growth was relatively higher than
269 other BDS studies used in the two phase systems,[29-32] an oil having high boiling
270 temperature, xylene (bp. 134-139 ^{o}C), was selected as the oil phase. Although DBT
271 containing xylene phase ceased the growth of the microorganism when it is supplied
272 in the two phase system at 40% (v/v), equilibrium between xylene concentration,
273 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.[31, 32] Since the solubility of DBT is 0.005 mM in water,[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 *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 *S. solfataricus* P2.

3. Experimental

3.1 Chemicals

S. solfataricus was obtained as a powder from American Type Culture Collection (ATCC$^{(R)}$ 35091^{TM}). DBT (99%) was obtained from Acros Organics, DBT-sulfone (97%) was from Sigma Aldrich, 4,6-Dimethyldibenzothiophene (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 $CaCl_2.2H_2O$, 1.3 g NH_4Cl, 0.25 g $MgCl_2.6H_2O$, 0.28 g KH2PO4 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[16] was prepared with 25 g l^{-1} EDTA, 2.14 g l^{-1} $ZnCl_2$,

299 2.5 g l^{-1} $MnCl_2.4H_2O$, 0.3 g l^{-1} $CoCl_2.6H_2O$, 0.2 g l^{-1} $CuCl_2.2H_2O$, 0.4 g l^{-1}

300 $NaMoO_4.2H_2O$, 4.5 g l^{-1} $CaCl_2.2H_2O$, 2.9 g l^{-1} $FeCl_3.6H_2O$, 1.0 g l^{-1} H_3BO_3, 0.1 g

301 l^{-1} KI. Minimal medium[17] was adjusted to pH 3 and supplemented with yeast extract

302 (0.15% w/v) and glucose (20 g l^{-1}). Initial stocks of *S. solfataricus* culture were

303 initially made by using minimal medium and kept at -80 ^{o}C as 10% glycerol stocks of

304 1 ml aliquots. Cell cultivation was carried out at 78 °C in 250 ml flasks containing

305 100 ml of medium with 160 rpm shaking.

306 3.3 Carbon utilization

307 SFM culture medium was employed as the base medium and was supplemented

308 with D-arabinose, ethanol, D-glucose and D-mannitol as different sources of

309 carbon to a final concentration of 2 g l^{-1}. To find out the optimum sulfur free

310 growth condition, various concentrations of the most effective carbon source,

311 glucose, was added on SFM culture medium at concentrations of 2, 5, 10, 15 and 20

312 g l^{-1}. Data are represented as the means of triplicate cultures ± standard error.

313 3.4 Sulfur utilization

314 The ability of *Sulfolobus solfataricus* P2 to utilize organic and inorganic sulfur

315 sources was investigated. Several organic and inorganic sulfur compounds

316 including DBT, BT, DBT-sulfone, 4,6-dimethyldibenzothiophene (4,6-DMDBT),

317 elemental sulfur, sodium sulfide, sodium sulfate, potassium persulfate and potassium

318 disulfite were added with an initial concentration of 0.3 mM to SFM culture as the

319 sole source of sulfur. However, there is a trace amount of sulfur contaminating from

320 the stocks of the culture, which were first prepared using minimal medium. Sulfur

321 content originating from the stocks of *S. solfataricus* in SFM was measured using

322 inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin-Elmer,

USA) as described in a previous study.[18] 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 OD600 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) Na_2CO_3, 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.

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473

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

	growth rate (h^{-1})	**maximum cell density ($g\ l^{-1}$)**
2 $g.L^{-1}$ glucose	0.0164 ± 0.0006	0.149 ± 0.008
5 $g.L^{-1}$ glucose	0.0192 ± 0.0004	0.148 ± 0.003
10 $g.L^{-1}$ glucose	0.0217 ± 0.0006	0.139 ± 0.002
15 $g.L^{-1}$ glucose	0.0276 ± 0.0014	0.149 ± 0.005
20 $g.L^{-1}$ glucose	0.0345 ± 0.0011	0.199 ± 0.003

484

485 **Table 2.** Various organic sulfur compound utilization by *S. solfataricus* P2 in SFM
486 medium
487

	growth rate (h^{-1})	**maximum cell density ($g\ l^{-1}$)**
4.6 DMDBT	0.0172 ± 0.0011	0.423 ± 0.031
DBT-sulfone	0.0179 ± 0.0056	0.281 ± 0.011
BT	-	0.192 ± 0.009
DBT	-	0.183 ± 0.004

488
489
490

Table 3. Various inorganic sulfur compound utilization by *S. solfataricus* P2 in SFM medium

	growth rate (h^{-1})	**maximum cell density ($g\ l^{-1}$)**
Elemental S	0.0165 ± 0.0012	0.586 ± 0.016
Sodium sulfite	0.0226 ± 0.0006	0.628 ± 0.053
Potassium disulfite	0.0254 ± 0.0005	0.623 ± 0.008
Sodium sulfate	0.0220 ± 0.0008	0.651 ± 0.005
Potassium persulfate	0.0222 ± 0.0003	0.651 ± 0.001

Table 4. Utilization of increasing DBT concentrations by *S. solfataricus* P2

	growth rate (h^{-1})	**maximum cell density ($g\ l^{-1}$)**
0.1 mM DBT	0.0122 ± 0.0014	2.19 ± 0.28
0.2 mM DBT	0,0061 ± 0.0011	2.13 ± 0.11
0.3 mM DBT	0.0020 ± 0.0002	0.87 ± 0.01
0.4 mM DBT	-	0.73 ± 0.01
yeast medium (control)	0.0149 ± 0.0010	1.57 ± 0.05

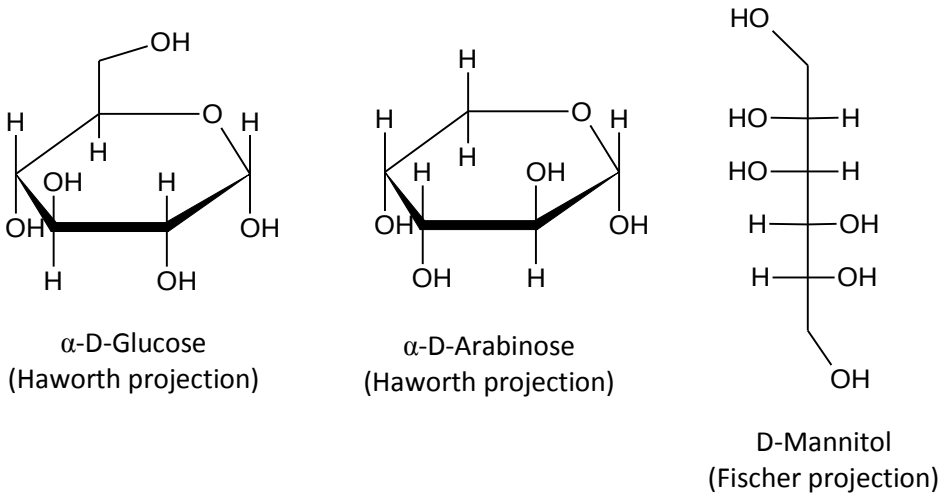
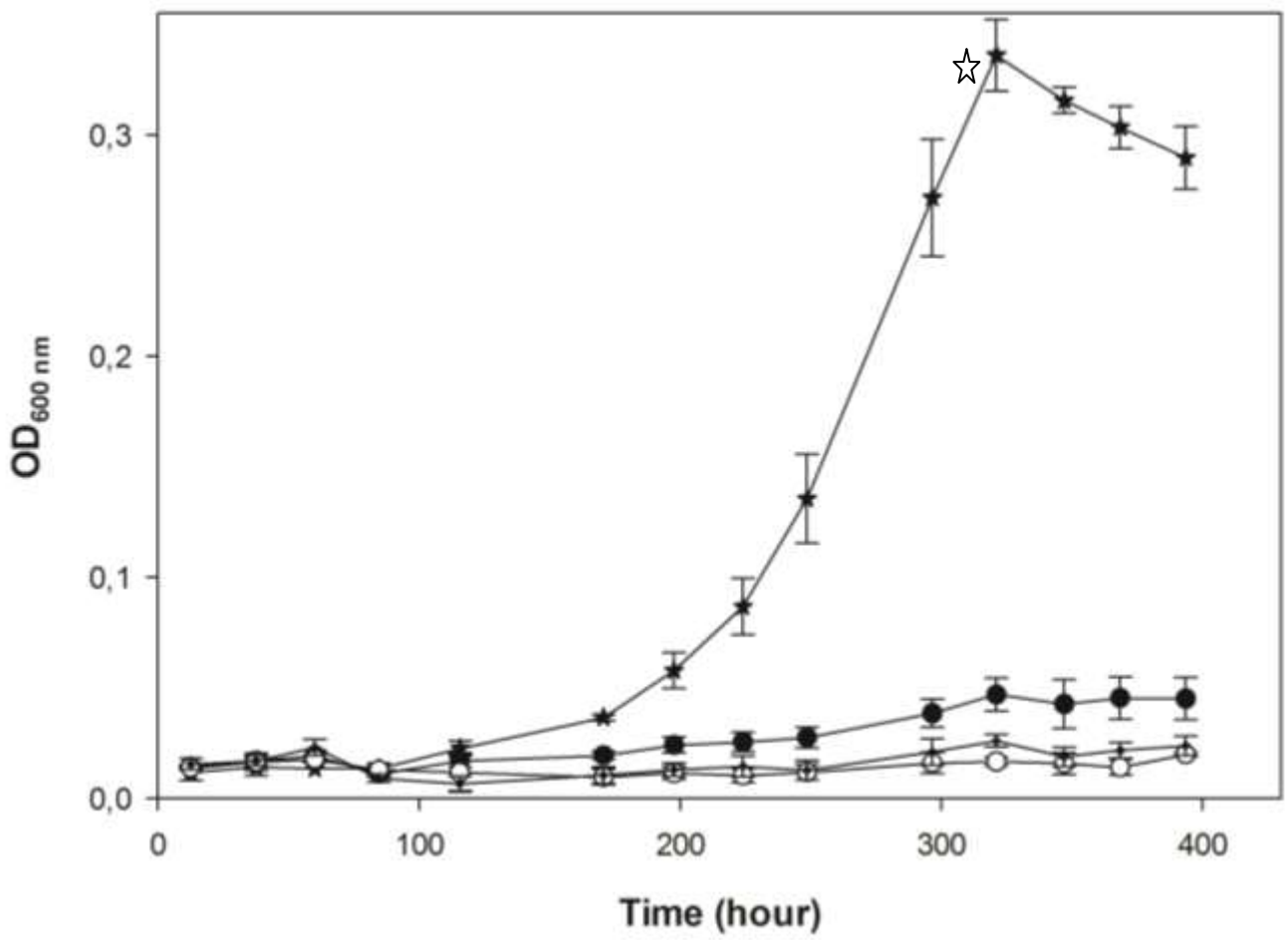


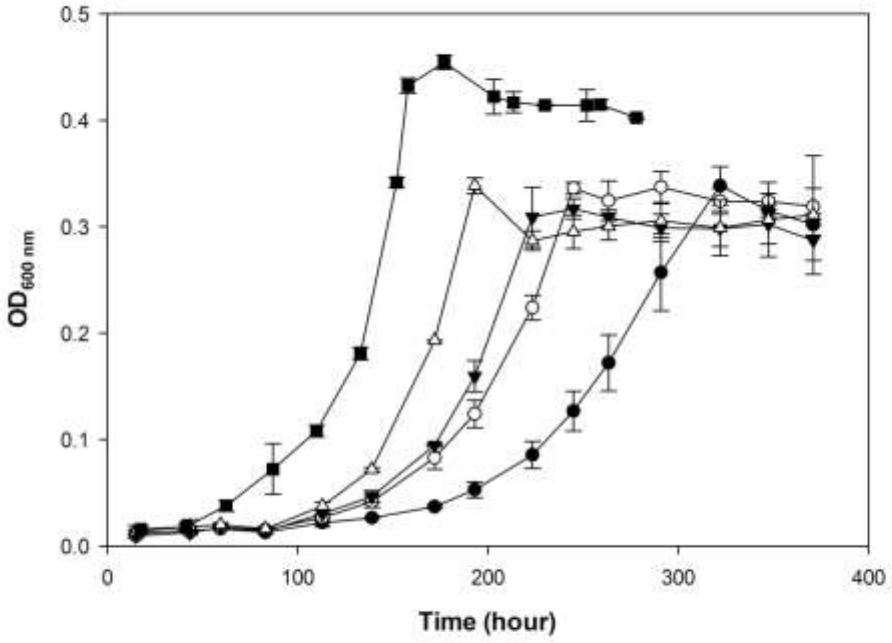
Figure 1: Some of the carbon sources used in the study. Molecular structures of D-glucose, D-arabinose and D-mannitol are shown.



516
517

518 **Figure 2:** Effects of different carbon compounds (concentrations of 2 g l^{-1}) on the
519 growth of *S. solfataricus* P2 in SFM medium. (o) D-mannitol, (●) D-arabinose, (+)
520 ethanol, (⋆) D-glucose. The symbol star represents the highest growth rate observed
521 for D-glucose.

522

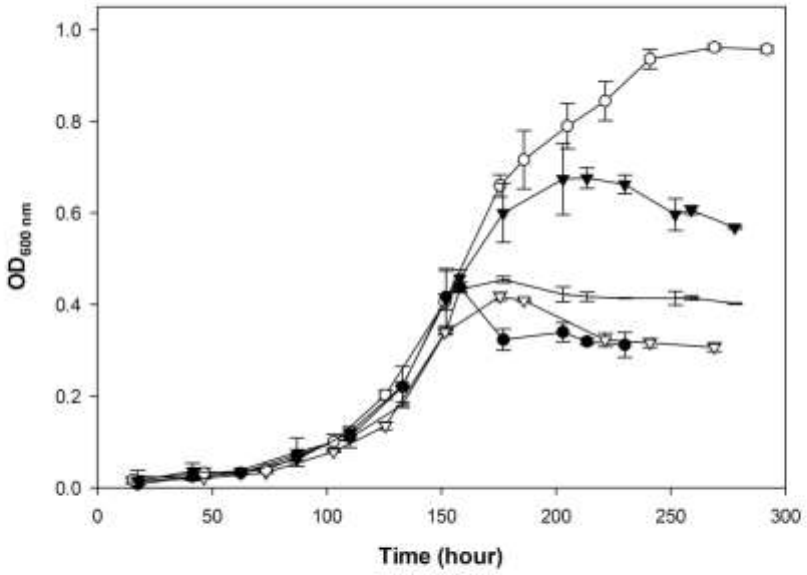


523

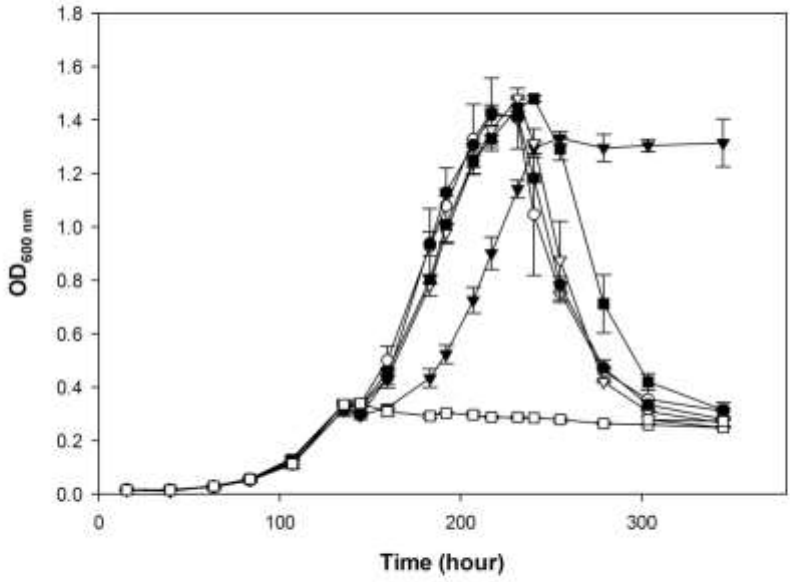
524 **Figure 3:** Glucose gradients from 2 g l^{-1} to 20 g l^{-1} were performed in SFM

525 medium. (●) 2, (○) 5, (▼) 10, (△) 15 and (■) 20 g l^{-1} glucose

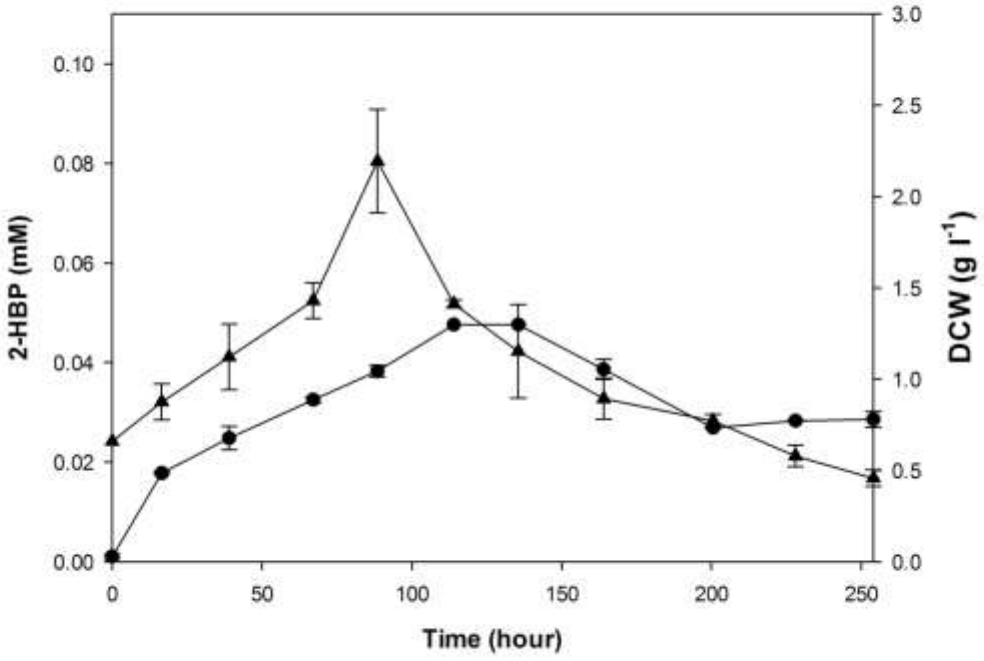
526



527
528 **Figure 4:** Growth of *S. solfataricus* P2 in the presence of 0.3 mM organic sulfur
529 sources in SFM medium supplemented with 20 g l^{-1} glucose. (●) BT, (○) 4-6
530 Dimethyldibenzothiophene, (▼) DBTsulfone, (▽) DBT and (-) SFM-only
531 medium. Sulfur sources were supplemented to the growing cultures at OD600
532 near 0.4
533



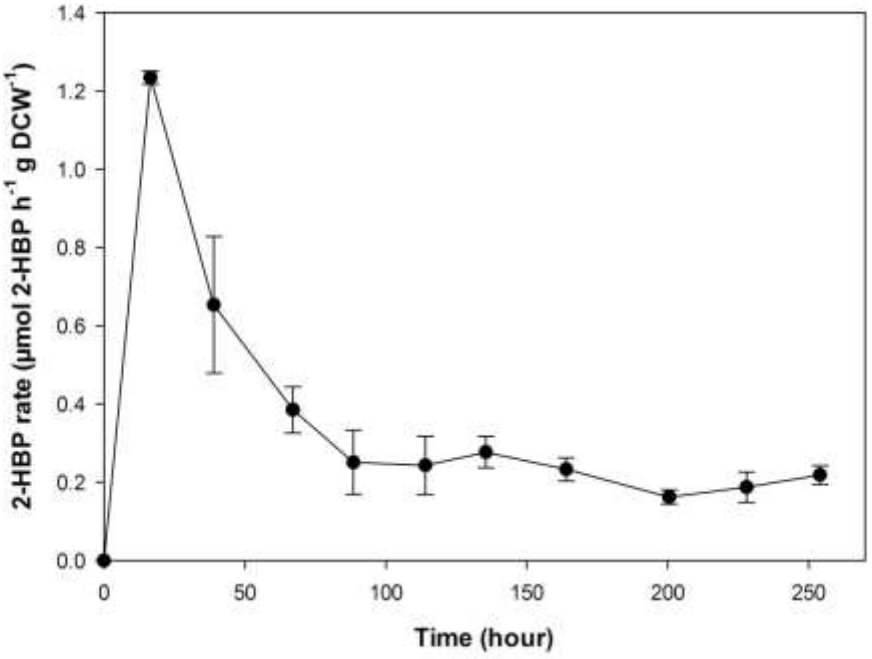
534
535 **Figure 5:** Growth of *S. solfataricus* P2 in the presence of 0.3 mM inorganic sulfur
536 sources in SFM supplemented with 20 g l^{-1} glucose. (▼) elemental sulfur, (○)
537 sodium sulfite, (■) sodium sulfate, (▽) potassium persulfate, (●) potassium
538 disulfite and (□) SFM-only medium. Sulfur sources were supplemented to the
539 growing cultures at OD_{600} near 0.4
540



541
542 **Figure 6:** Formation of 2-HBP by the growing cells of *Sulfolobus solfataricus* P2.

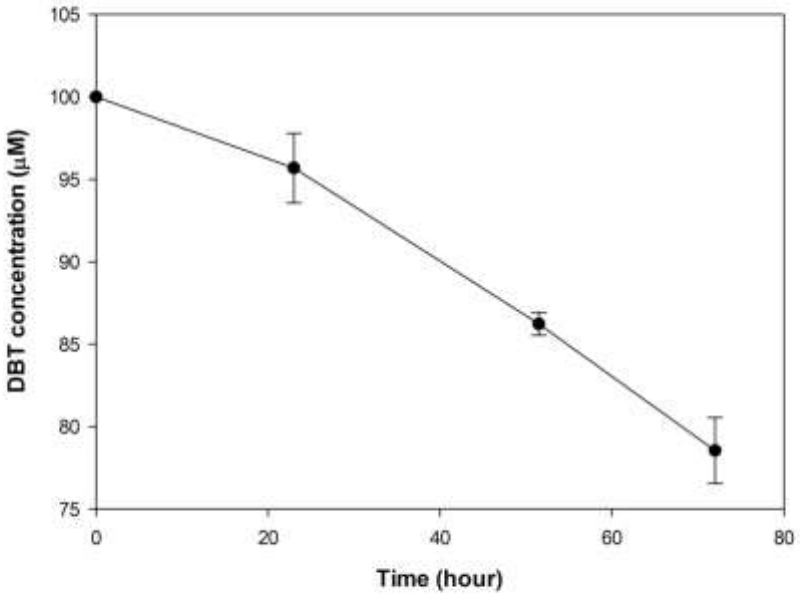
543 DBT was supplemented to growing cultures in minimal medium at 0.66 g dry cell l^{-1}.

544 (▲) DCW, (●) 2-HBP



545
546 **Figure 7:** The time course of specific production rate of 2-HBP from 0.1 mM DBT

547 by *Sulfolobus solfataricus* P2



548
549 **Figure 8:** Consumption of DBT. Experiments were performed in minimal medium
550 containing 40% (v/v) xylene

551