1 **Revisit to the biodesulfurization capability of hyperthermophilic**

2 **archeon *Sulfolobus Solfataricus* P2 revealed DBT consumption**

3 **by the organism in an oil/water two-phase liquid system at high**

4 **temperatures**

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29   **Running head:** Biodesulfurization capability of *S. solfataricus*

30

31

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 $^{\circ}$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.

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

74 complementary to hydrodesulfurization requires an additional cooling process of the

75 fuel to ambient temperature following HDS. This additional cooling process causes

76 an economical burden when used for large scale fossil fuel desulfurization. Thus,

77 hyperthermophilic microbial desulfurization is desirable and makes the crude oil

78 biodesulfurization process more feasible due to low viscosity of the crude at high

79 temperature.[3]

80 There were various attempts to use hyperthermophiles in biodesulfurization to date,[12-

81 15] yet most of these studies were able to clearly delineate the pyritic sulfur

82 desulfurization, but lack to show reliable sufficient amounts of organic sulfur removal

83 efficiency. A study that undertook the usage of a hyperthermophilic *Sulfolobus*

84 *acidocaldarius* in DBT utilization revealed the oxidation of sulfur present in DBT to

85 sulfate at 70 $^{\circ}$C.[13] Unfortunately, that study did not include DBT degradation at high

86 temperatures in the absence of microorganism[13], therefore the obtained rate of

87 desulfurization could not represent the real biodesulfurization rate. Another attempt to

88 study heterocyclic organosulfur desulfurization using a thermophile, *Sulfolobus*

89 *solfataricus* DSM 1616[15] at 68 $^{\circ}$C showed DBT self-degradation in the absence of

90 microorganism at high temperatures, thus no substantial DBT utilization could be

91 observed. This study clearly pointed the difficulty to use DBT model compound at

92 high temperatures in biodesulfurization by *S. solfataricus*.[15] Nonetheless, the same

93 study showed the oxidation of thiophene-2-carboxylate by *S. solfataricus*, [15] therefore

94 organic sulfur desulfurization molecular mechanism had shown to be present in this

95 hyperthermophile, and further investigations are necessary to optimize the conditions

96 for better organic sulfur removal with possibly a different *Sulfolobus* strain, which

97 might lead to better efficiency for desulfurization.

98 Hyperthermophiles are isolated mainly from water containing volcanic areas such as

99   solfataric fields and hot springs in which they are unable to grow below 60 °C.

100   *Sulfolobus solfataricus* P2 belonging to archaebacteria grows optimally at

101   temperatures between 75 and 85 °C and at low pHs between 2 and 4, utilizing a wide

102   range of carbon and energy sources.

103   This paper describes the potential of a hyperthermophilic archaeon, *S. solfataricus* P2,

104   to utilize several inorganic and organic sources of sulfur for growth in various

105   conditions, and shows *S. solfataricus* P2's ability to remove sulfur from DBT via

106   the sulfur-selective pathway even under high temperatures with the elimination of

107   DBT self-degradation. To the best of our knowledge, this is the first report

108   showing the DBT desulfurization kinetics analysis of *S. solfataricus* P2.

109

110   **2. Results and discussion**

111   2.1 Carbon source influence on the growth of *S. solfataricus* P2

112   The ability of *S. solfataricus* P2 to use several sources of carbon was investigated.

113   Four types of carbon sources have been applied to the SFM medium: D-glucose,

114   D-arabinose, D-mannitol (Figure 1) and ethanol. All these experiments have been

115   carried out employing 2 g l^{-1} as the initial concentration of carbon source. Figure 2

116   shows the effects of different sources of carbon on archaeal growth. The highest

117   growth rate, 0.0164 h^{-1}(60.9 h), and the maximum biomass density, 0.149 g dry

118   weight l^{-1}, were observed when D-glucose was employed as a carbon source

119   (Figure 2). On the other hand, D-arabinose, D-mannitol and ethanol (at a

120   concentration of 2 g l^{-1}) did not support the growth (Figure 2). Our data in Figure

121   2 clearly showed that glucose is a better carbon source for the growth of *S.*

122   *solfataricus* P2 compared to the tested other carbon sources. *S. solfataricus* harbors

123   a semi-phosporylative Entner-Doudoroff (ED) pathway for sugar metabolism.[19, 20]

124   Since D-glucose is the first metabolite necessary to initiate glycolysis, it is rather

125   expected to observe better D-glucose utilization than the other sugars. For both D-

126   and L-arabinose a well-defined pentose mechanism exists in *S. solfataricus*.[19] Both

127   pentose mechanisms may include intermediates that are not heat stable, thus these

128   products may get degraded while enough ATP gets accumulated to allow cells to

129   survive. As presented with a recent study, unstable intermediate metabolites exist

130   for semi-phosporylative ED pathway in glucose metabolism for hyperthermophiles

131   that grow at extreme temperatures,[20] therefore similar type of unstable intermediate

132   production in the pentose mechanism may prevent the growth of *S. solfataricus*

133   cells under scarce sugar supplies.

134   To further determine the optimum growth condition of *S. solfataricus* P2 in SFM

135   medium when glucose is the source of carbon, various concentrations of glucose

136   ranging from 2 g l^{-1} to 20 g l^{-1} on SFM culture were employed. The results

137   revealed that the highest growth rate; 0.0339 h^{-1} (29.5 h) and biomass concentration;

138   0.157 g l^{-1} were obtained when 20 g l^{-1} of glucose was used (Figure 3). It can be

139   affirmed that the higher the glucose concentration, the higher the growth rate is

140   (Table 1). Figure 3 also indicates that with increasing concentrations of glucose,

141   enhanced growth rate was observed, and the time required to reach the maximum

142   biomass value was decreased; however the maximum cell densities obtained with

143   increasing concentrations of glucose were similar for all of the concentrations

144   (ranging from 0.14 to 0.157 g DCW l^{-1}). At the same time, the lag time decreased

145   with the highest concentration of glucose application, and cells reached to the

146   stationary phase faster as the concentration of glucose was increased. One explanation

147   for the observed increased rate for the growth with higher glucose concentration

148   might due to allowing cells steadily obtain all the necessary intermediate metabolites,

149 even some of them get degraded under high temperatures,[20] still excess amounts for

150 productive glycolytic cycles would be enough for cells to proliferate. Although, an

151 acceptable growth profile was observed when glucose was employed as the

152 carbon source; overall, in SFM medium, presence of glucose was not sufficient to

153 obtain an optimal growth, additional micronutrients were necessary to optimize the

154 growth conditions.

155 2.2 Organic sulfur compounds utilization

156 The ability of *S. solfataricus* P2 to utilize organic sulfur compounds was evaluated

157 toward 4,6- DMDBT, DBT sulfone, DBT and BT. Each was acted as a sole source

158 of sulfur for the growth with an initial concentration of 0.3 mM in SFM culture

159 except the presence of trace amounts of sulfur originating from the culture stocks.

160 ICP-OES analysis revealed the presence of 0.00168 ± 0.0008 g l^{-1} sulfur in the 100 ml

161 control flasks. Unless otherwise noted, all the cultivation experiments were done in

162 the same manner, and their initial sulfur contents were estimated to be similar to the

163 initially determined value. Also for all of the growths, 20 g l^{-1} of glucose was

164 employed as a carbon source in SFM medium. The effects of the organic sulfur

165 compounds on the growth are shown in Figure 4. When the cultures were incubated

166 initially with DBT, DBT-sulfone, 4,6-DMDBT and BT, there were no archaeal

167 growth (data not shown). Instead of employing organic compounds in the

168 beginning of the growth, each organic sulfur compound was separately added into

169 SFM medium after a moderate optical density (OD between 0.35 and 0.4, around

170 the midst of log phase during *S. solfataricus* P2 growth) was attained. Thus,

171 supplementation of organic compounds in this way enabled *S. solfataricus* P2 cells

172 to grow well on media containing DBT-sulfone and 4,6-DMDBT as the sole

173 sources of sulfur; but addition of BT resulted abrupt interruption of cell growth, and

174 subsequently led the cells to death (Figure 4). DBT addition, on the other hand,

175 progressively ceased the growth of the cells (Figure 4). Maximum biomass densities

176 and specific growth rates are given in Table 2. Maximum cell density was achieved

177 with 4,6-DMDBT, yielding 2.5 times higher cell density compared to that of the

178 control. DBT-sulfone presence enabled cells to achieve 1.4 times higher cell

179 density with respect to the control. These results revealed that *S. solfataricus* P2

180 can utilize organic sulfur compounds containing DBT and its derivatives; but, even

181 among them, it has certain preference to some types of organic molecules than the

182 others. Results indicated that *S. solfataricus* P2 cannot utilize BT. Since DBT

183 and BT desulfurization pathways were shown to be different for various

184 desulfurizing bacteria,[16, 21] it can be concluded that *S. solfataricus* P2 has a

185 metabolic pathway specific for DBT and its derivatives.

186 2.3 Inorganic sulfur compounds utilization

187 To compare the effects of the organic and inorganic sulfur sources on growth, 0.3

188 mM inorganic sulfur sources as a sole sulfur source; elemental sulfur, sodium

189 sulfite, sodium sulfate, potassium persulfate and potassium disulfite were

190 employed into the SFM medium at OD_{600} around 0.32. Growth curve patterns

191 of cultures containing inorganic sulfur sources were similar except for the

192 elemental sulfur case (Figure 5). All the growth curves reveal a short stationary

193 period after supplementation of the inorganic sulfur compounds, suggesting a

194 certain adaptation time for the cells to the new nutrient environment. This

195 adaptation period may correlate to the immediate uptake of inorganic sulfur

196 molecules by the cells. A logarithmic enhancement in the growth followed by this

197 short stationary period shows that *S. solfataricus* P2 utilizes the supplied

198 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

224   compounds in SFM culture medium. Since DBT has been used as the model

225   molecule of the thiophenic compounds present in fossil fuels, we aimed to optimize

226   DBT utilization levels of *S. solfataricus* P2 by changing the growth medium

227   conditions. Addition of yeast extract in the minimal medium significantly enhanced

228   the utilization levels of DBT by *S. solfataricus* P2. The effect of different

229   concentrations of DBT was tested in the growth of S. *solfataricus* P2 (Table 4); and

230   with 0.1 mM DBT supplementation, cell density was enhanced significantly

231   compared to the control, where no DBT was added in the minimal medium, and to

232   the increasing DBT concentrations. Higher amounts of DBT usage showed

233   significantly lower maximum cell density; and therefore 0.1 mM of DBT was

234   used in our DBT desulfurization kinetics studies (Table 4). A continuous growth

235   was observed until 89 h with a simultaneous production of 2-HBP, which was

236   determined by both Gibbs assay and GC (Figure 6). It was observed that DBT

237   concentration decreased sharply under abiotic conditions (data not shown). Earlier

238   work also revealed DBT to be unstable at higher temperatures in aqueous

239   environment.[15] However, even under these conditions, desulfurization activity was

240   observed in growing cultures, and is estimated to be 1.23 µmol 2-HBP h^{-1} g DCW^{-}

241   ^{1}. Specific production rate of 2-HBP was decreased sharply after 16.5 h as can be

242   seen in Figure 7. Similar abrupt decrease in the production rate of 2-HBP was

243   observed earlier in most of the BDS studies,[22-25] and was explained by the

244   production of HBP in the medium causes substrate inhibition type of enzyme

245   kinetics (26). Although 93% of DBT depletion was observed within 39 h, 2-HBP

246   production was continued to increase up until 114 h to a concentration of 47.6

247   µM. Growth of *S. solfataricus* P2 stopped near the maximum levels of 2-HBP was

248   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

274   effective DBT biodesulfurization when applied to industrial usage.

275   Two oil phase system has been used for enhancing the poor solubility of many

276   organic compounds in aqueous cultures.[31, 32] Since the solubility of DBT is 0.005

277   mM in water,[32] aqueous/apolar culture system has an advantage on the

278   biodesulfurization of DBT and its derivatives.

279   In conclusion, since biodesulfurization done under high temperatures offers a

280   potential for an alternative/complementary method for lowering the sulfur content

281   of fossil fuels, in that respect, hyperthermophilic *S. solfataricus* P2 with its

282   potential DBT-desulfurization ability can serve as a model system for the efficient

283   biodesulfurization of fossil fuels. Further molecular biology studies for the

284   characterization of the genes responsible for DBT desulfurization, undertaken already

285   by our group, will enable to delineate the exact BDS mechanism of *S. solfataricus*

286   P2.

287   **3. Experimental**

288   3.1 Chemicals

289   *S. solfataricus* was obtained as a powder from American Type Culture Collection

290   (ATCC[(R)] 35091[TM]). DBT (99%) was obtained from Acros Organics, DBT-sulfone

291   (97%) was from Sigma Aldrich, 4,6-Dimethyldibenzothiophene (97%), elemental

292   sulfur (99%), were from ABCR, DMF was from Riedel-de Haën. All other reagents

293   were of the highest grade commercially available.

294   3.2 Culture media and growth conditions

295   Sulfur-free mineral (SFM) medium was prepared by dissolving 70 mg of

296    $CaCl_2.2H_2O$, 1.3 g NH_4Cl, 0.25 g $MgCl_2.6H_2O$, 0.28 g $KH2PO4$ and 0.5 ml trace

297   elements solution in 1 l of milli-Q water, and this mix was adjusted to pH 3 with

298   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,

323   USA) as described in a previous study.[18] In all of these media, 20 g l^{-1} of glucose

324   was used as the sole source of carbon. SFM culture containing only the carbon

325   source (20 g l^{-1} of glucose) was used as a control. Stock solutions of organic sulfur

326   compounds, DBT, BT, 4,6-DMDBT and DBT-sulfone were dissolved in N,N-

327   dimethylformamide (100 mM). In all of these experiments, organic sulfur

328   compounds were added to the growth culture after a certain exponential growth

329   was achieved, corresponding to an OD_{600} (optical density at 600 nm) value in

330   between 0.35 and 0.4. Data are represented as the means of triplicate cultures ±

331   standard error.

332   For desulfurization kinetics assay, minimal medium supplemented with 0.1 mM

333   DBT, 0.15% w/v yeast extract and glucose (20 g l^{-1}) was used in the presence and

334   absence of 40% (v/v) xylene. Cells grown at the mid-log phase (OD_{600} being 1.5)

335   were supplemented with DBT or DBT dissolved in xylene in a two-state oil phase.

336   3.5 Analytical methods

337   Cell densities were measured at the 600 nm wavelength using a Shimadzu UV

338   visible spectrophotometer (model UV-1601). A correlation between OD_{600} and dry

339   cell weight (DCW) was done to determine the concentration of cells. One unit of

340   optical density corresponded to 0.44 g DCW l^{-1}.

341   3.6 Analysis of organic sulfur compounds and metabolites

342   For gas chromatography (GC) experiments, aliquots of the culture during the

343   course of bacterial growth were acidified below pH 2.0 with 1 N HCl, then culture

344   was extracted with equal volumes of ethyl acetate during a 5 min vortex and 10 min

345   centrifugation at 2000 rpm. For the two-phase system, xylene fractions were directly

346   used for DBT quantification. 2 µl of the organic fraction was used for the detection

347   of DBT and 2-HBP by using a GC (HP-Agilent Technologies 6890N GC Systems,

348   USA) equipped with a flame ionization detector. Agilent JW Scientific DB-5

349   capillary 30.0 m × 0.25 mm × 0.25 µm column was used for the measurements.

350   Temperature was set to 50 °C for 5 min followed by a 10 °C min^{-1} rise up to

351   280 °C and kept at this temperature for 5 min. Injector and detector temperatures

352   were both maintained at 280 °C. Quantification of DBT and 2-HBP were performed

353   using standard curves with a series of dilutions of the pure DBT and 2-HBP

354   compounds as a reference. All the reaction mixtures were prepared as triplicates.

355   3.7 Gibb's assay / Desulfurization assay

356   The Gibb's assay was used in conjunction with GC analyses to detect and quantify

357   the conversion of DBT to 2-HBP produced by the *Sulfolobus solfataricus* P2 in the

358   culture media lacking xylene. The assay was carried out as follows: 1 ml of culture

359   was adjusted to pH 8.0 with 10% (w/v) Na_2CO_3, then 20 µl of freshly prepared

360   Gibb's reagent (2,6-dicholoroquinone-4-chloroimide, 5 mM in ethanol) was added.

361   The reaction mixtures were allowed to incubate for 60 min at 30 °C for color

362   development. The mixtures were then centrifuged at 5000 rpm for 10 min to remove

363   cells, and absorbance of the supernatant was determined at 610 nm (UV 1601,

364   Shimadzu, Japan). Concentration of produced 2-HBP from the Gibb's assay results

365   was determined from the standard curve obtained by different concentrations of pure

366   2-HBP. Results correspond to the means of three different experiments with the

367   standard errors included.

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372   Technical University internal funds, Turkey.

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474

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

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	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

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

	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

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

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	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

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499 **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	$0,0061 \pm 0.0011$	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

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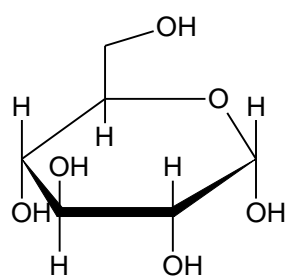
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(Haworth projection)
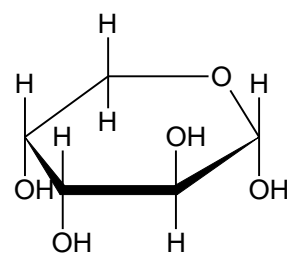
α-D-Arabinose
(Haworth projection)
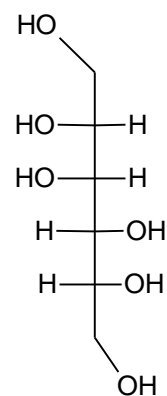
D-Mannitol
(Fischer projection)

513   **Figure 1:** Some of the carbon sources used in the study. Molecular structures of D-

514   glucose, D-arabinose and D-mannitol are shown.

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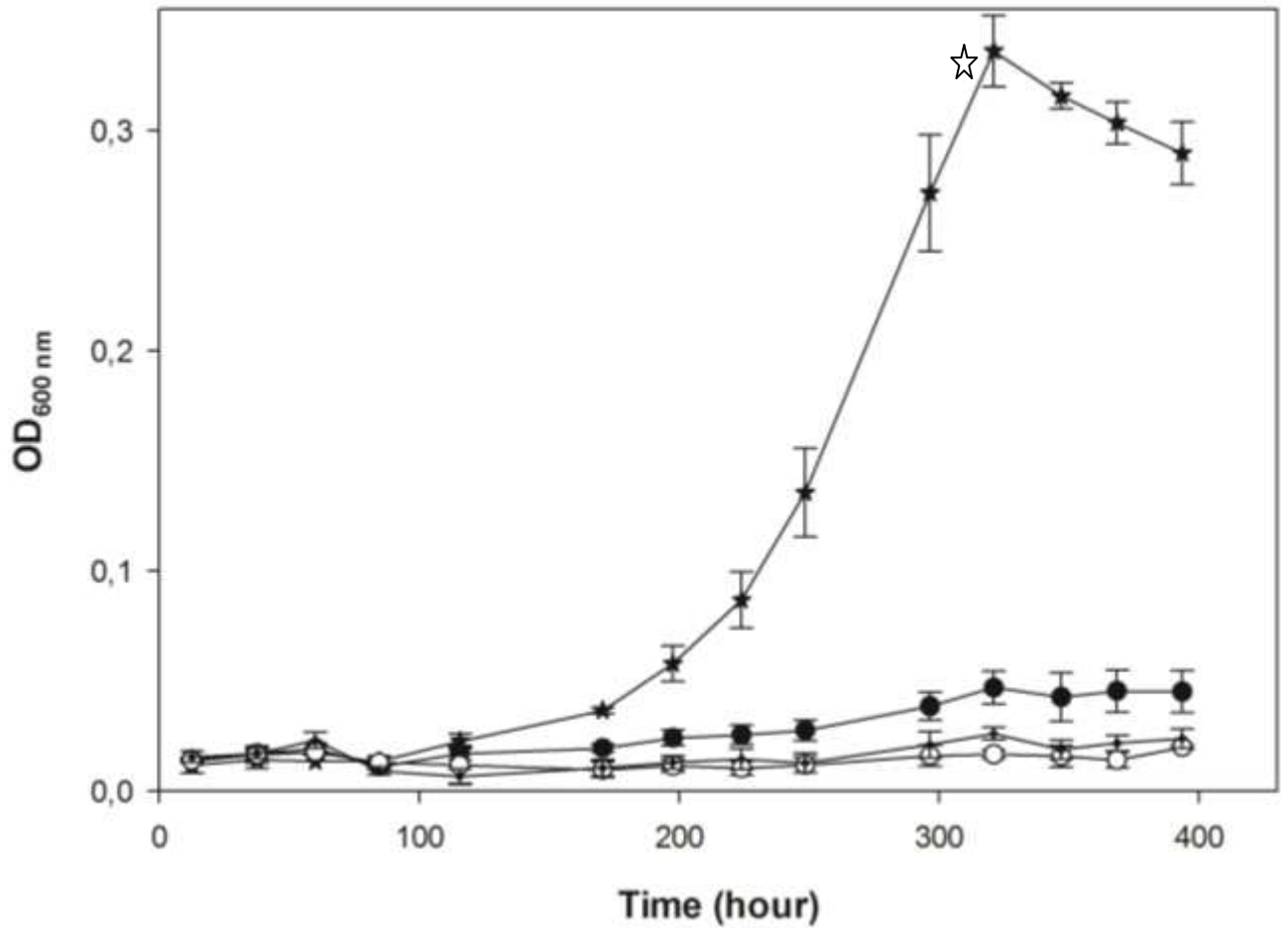
Figure 2: Effects of different carbon compounds (concentrations of 2 g l^{-1}) on the

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.

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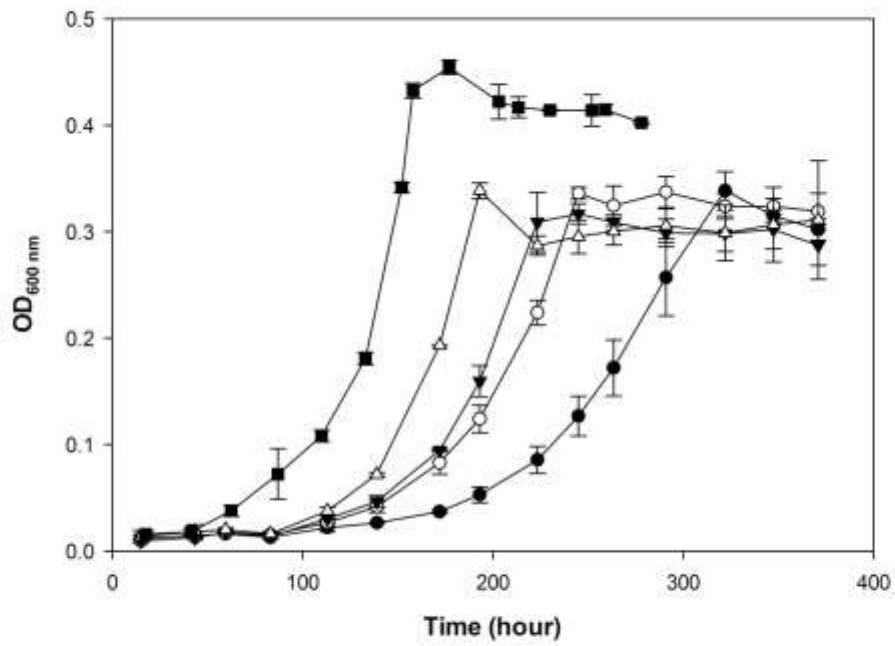
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Figure 3: Glucose gradients from 2 g l^{-1} to 20 g l^{-1} were performed in SFM

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

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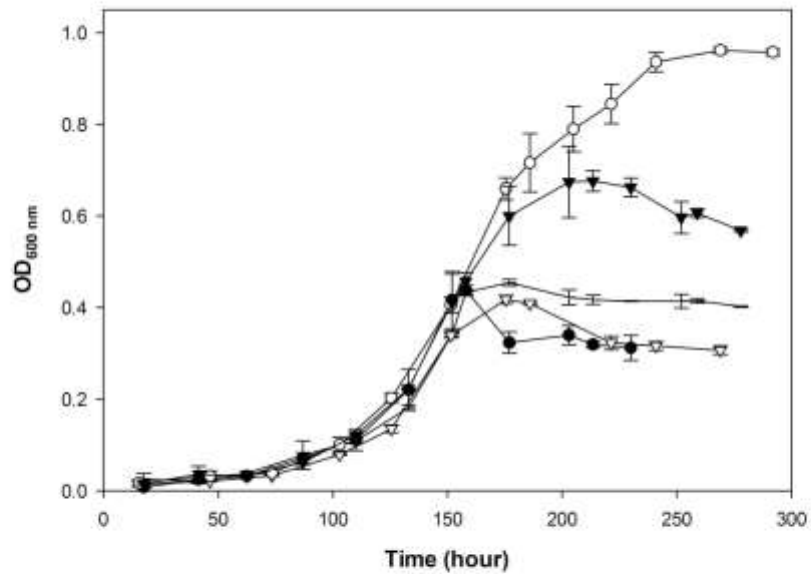
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528 **Figure 4:** Growth of *S. solfataricus* P2 in the presence of 0.3 mM organic sulfur

529 sources in SFM medium supplemented with 20 g l^{-1} glucose. (●) BT, (O) 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

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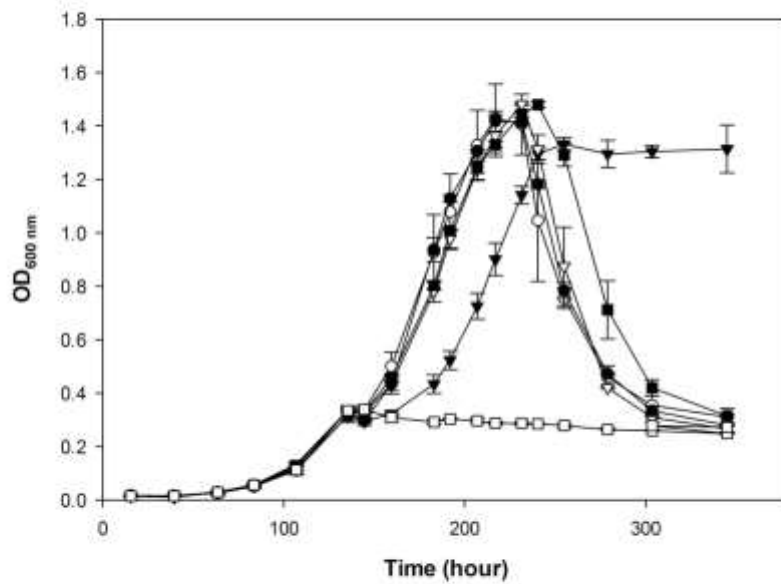
Figure 5: Growth of *S. solfataricus* P2 in the presence of 0.3 mM inorganic sulfur sources in SFM supplemented with 20 g l^{-1} glucose. (▼) elemental sulfur, (O) sodium sulfite, (■) sodium sulfate, (▽) potassium persulfate, (●) potassium disulfite and (□) SFM-only medium. Sulfur sources were supplemented to the growing cultures at OD600 near 0.4
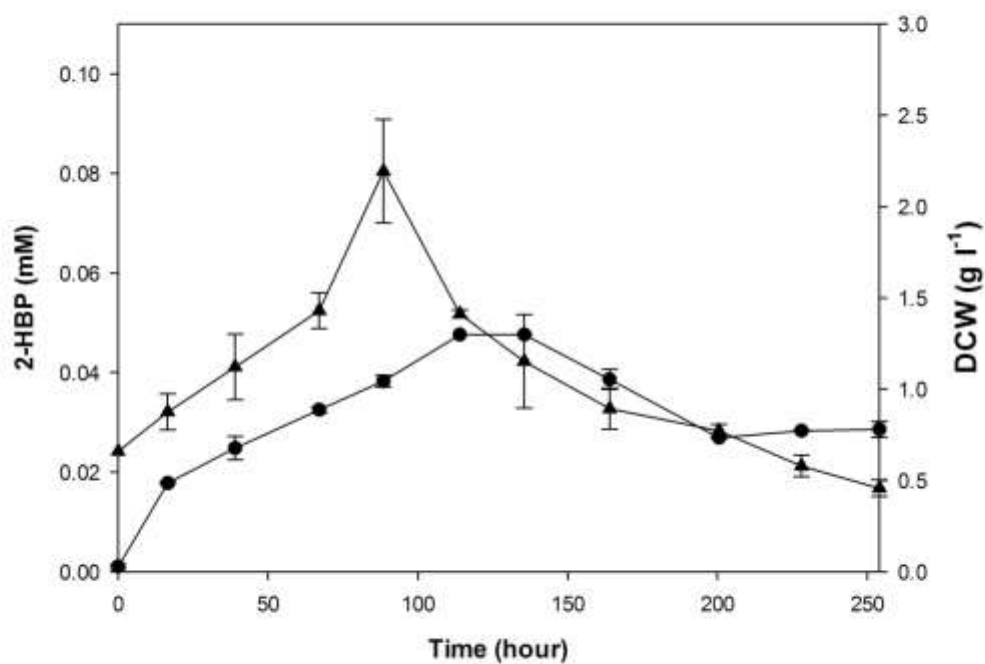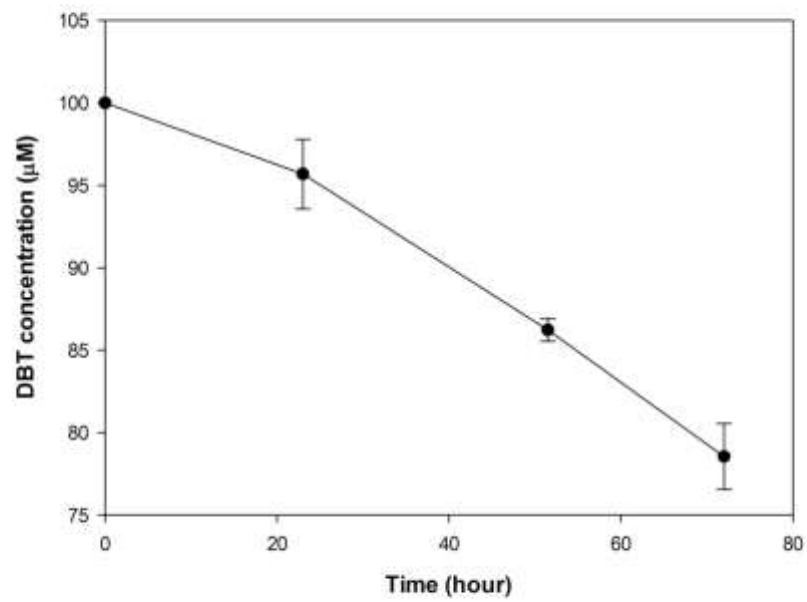
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542   **Figure 6:** Formation of 2-HBP by the growing cells of *Sulfolobus solfataricus* P2.

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

544   (▲) DCW, (●) 2-HBP
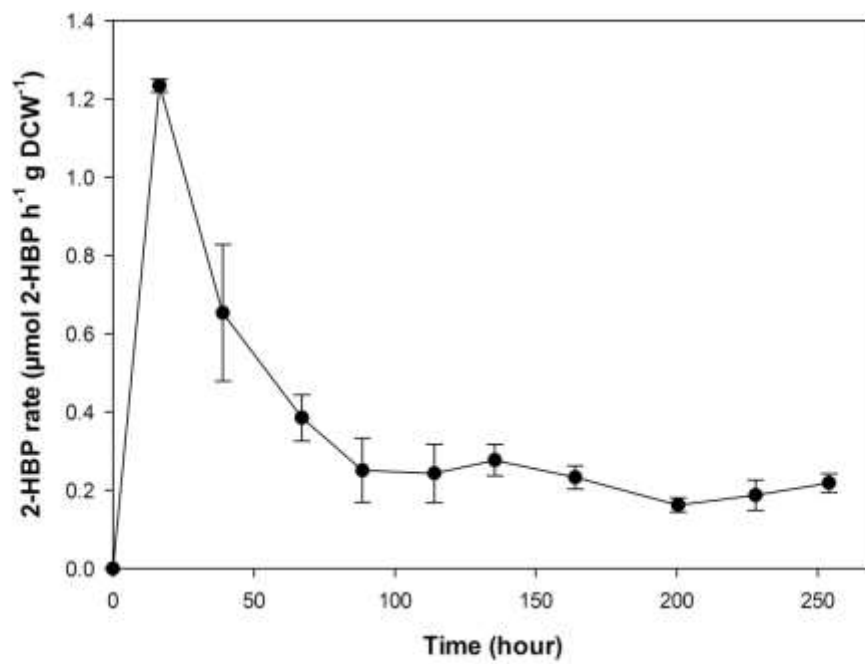
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546   **Figure 7:** The time course of specific production rate of 2-HBP from 0.1 mM DBT

547   by *Sulfolobus solfataricus* P2

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549 **Figure 8:** Consumption of DBT. Experiments were performed in minimal medium

550 containing 40% (v/v) xylene

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