Biotechnology Letters **25:** 579–583, 2003.

2-Propanol degradation by *Sulfolobus solfataricus*

Helia Radianingtyas[1] & Phillip C. Wright[2,*]
[1]*Biochemical Engineering and Environmental Technologies Group, Department of Chemical and Process Engineering, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, UK*
[2]*Biological and Environmental Systems Group, Department of Chemical and Process Engineering, University of Sheffield, Mappin Street, Sheffield S1 3JD, UK*
Author for correspondence (Fax: +44 114 2227501; E-mail: p.c.wright@sheffield.ac.uk)

Received 21 November 2002; Revisions requested 19 December 2002; Revisions received 11 February 2003; Accepted 11 February 2003

Key words: biodegradation, biofilm, hyperthermophilic, immobilized, isopropanol

Abstract

Sulfolobus solfataricus used 2-propanol and 2-propanone (acetone) when grown in static cultures at 78 °C with or without glucose at 10 g l^{-1}. The presence of 3.92 g 2-propanol l^{-1} in both cases inhibited growth. However, acetone accumulation following 2-propanol depletion suggested that 2-propanol was co-metabolized via the acetone metabolic pathway. Glucose at 10 g l^{-1} increased 2-propanol and acetone utilization from 0.93 g l^{-1} to 1.77 g l^{-1} and from 0.11 g l^{-1} to 1.62 g l^{-1}, respectively. Without glucose, immobilized *S. solfataricus* cells increased the 2-propanol removal rate to 0.035 g l^{-1} h^{-1}, compared to 0.0012 g l^{-1} h^{-1} by its suspended counterpart. The results suggest the establishment of an immobilized reactor configuration is preferential for the treatment of high temperature solvent waste streams by this acidothermophilic Crenarchaeon.

Introduction

2-Propanol, or isopropyl alcohol (IPA), is the most widely used Volatile Organic Compound (VOC), whose production worldwide exceeds 1 million tonnes per annum (Harris 1991). In 1995 alone, 2-propanol atmospheric emissions in the UK resulted in 52 000 tonnes of waste (Derwent & Pearson 1997). Thus, regulation to control the 2-propanol discharge has made the treatment of this compound becoming increasingly necessary. Biological treatment of 2-propanol-containing waste streams is one option that offers economic advantages. Biological treatment of 2-propanol by mesophiles within aerobic (Bustard *et al.* 2001, Hwang *et al.* 1995) as well as anaerobic environments (Fox & Ketha 1996) has been reported. Furthermore, Taylor *et al.* (1980) proposed that within a mesophilic anaerobic environment, 2-propanol was biodegraded via an acetone metabolic pathway. In contrast, fewer studies on 2-propanol biodegradation at elevated temperatures have been done. Recently Bustard *et al.* (2002) successfully demonstrated the biodegradation of 2-propanol by the thermophile *Bacillus pallidus* at 60 °C. In this study, we investigated the capability of the hyperthermophilic Crenarchaeon *Sulfolobus solfataricus* to degrade 2-propanol at 78 °C. This sulphur-utilizing Archaeon from solfatara habitats grows at temperatures from 50 to 87 °C and pH 2–5.5 in a microaerobic environment, thus suggesting biotechnological potential.

Materials and methods

Microorganism and culture conditions

Sulfolobus solfataricus, courtesy of Prof D. A. Cowan (University of the Western Cape, South Africa), was cultivated aerobically at 78 °C as static cultures using medium ATCC 1304 which consisted of 1 g yeast extract, 1 g Casamino acids, 3.1 g KH_2PO_4, 2.5 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.25 g $CaCl_2 \cdot 2H_2O$, 1.8 mg $MnCl_2 \cdot 4H_2O$, 4.5 mg $Na_2B_4O_7 \cdot 10H_2O$, 0.22 mg $ZnSO_4 \cdot 7H_2O$, 0.05 mg $CuCl_2 \cdot 2H_2O$, 0.03 mg $Na_2MoO_4 \cdot 2H_2O$, 0.03 mg

$VOSO_4 \cdot 2H_2O$ and 0.01 mg $CoSO_4 \cdot 7H_2O$ in 1 l distilled water. The pH was adjusted to 4–4.2 with 5 M H_2SO_4 at room temperature. This medium was sterilized at 121 °C for 15 min prior to all experiments.

Experimental set up

To observe the effect of glucose concentrations on the growth (expressed as mg protein l^{-1} medium) of *S. solfataricus*, 25 ml flasks with Suba-seal stoppers containing 20 ml growth medium were used. Solvent utilization experiments were carried out in screw-cap tubes (10 ml working volume). Filter-sterilized glucose, 1-propanol, 2-propanol or 2-propanone (acetone) was aseptically supplied to the growth medium after sterilization. Immobilized cultures were prepared using Celite R-635 (Celite Co., USA) diatomaceous earth pellets as a support matrix in 20 ml growth medium. Suspended cultures in similar volumes were also set up in order to compare substrate utilization. Negative controls containing cell-free growth medium and target substrates were also set up in every experiment to differentiate biological utilization from non-biological degradation, evaporation, and/or adsorption by the support matrix. All experiments were performed in at least triplicate with data presented corrected for evaporation, non-biological degradation and absorption rates. All figures show the average values with standard deviations of less than 5%.

Analytical methods

Growth as suspended cultures was determined by measuring the whole-cell protein using the Lowry assay. 100 μl of homogenous samples were taken from the cultures and treated with the BioRad DC Protein assay kit (BioRad, UK) according to the manufacturer's instructions. Medium ATCC 1304 was used as a blank and bovine serum albumin was used to make protein standard curves. 2-Propanol and acetone were analysed using a GC as per Bustard *et al.* (2002). Samples, 0.5 μl, were injected into the injection port (250 °C) then carried by He at 11 ml min^{-1} through a Carbowax BP20 column to a FID detector (300 °C). The column was initially set up at 70 °C with a temperature ramp of 50 °C per min up to 150 °C.

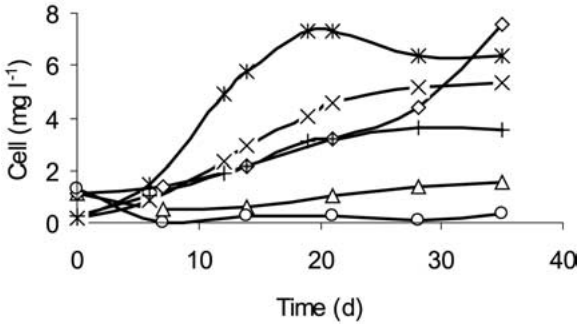

Fig. 1. Sulfolobus solfataricus cell growth (expressed as protein content) on: 20 g glucose l^{-1} (∗); 10 g glucose l^{-1} (×); 5 g glucose l^{-1} (+); 10 g glucose l^{-1} + 3.95 g acetone l^{-1} (◊); 10 g glucose l^{-1} + 3.92 g 2-propanol l^{-1} (△); and 10 g glucose l^{-1} + 4.02 g 1-propanol l^{-1} (○).

Results and discussion

Effect of glucose and solvents on suspended S. solfataricus *growth*

Experiments under batch conditions using a substrate concentration of 5 g l^{-1}, 10 g l^{-1} and 20 g l^{-1} confirmed that the higher the initial glucose concentration the greater the final biomass obtained (Figure 1). These results agree with those demonstrated previously by other researchers (Schiraldi *et al.* 1999). However, even at the highest glucose concentration supplied (i.e. 20 g l^{-1}), the biomass generation rate remained very low, reflecting an inefficient utilization of glucose. It has been hypothesized that this growth inhibition was due to low molecular weight ionic catabolic compounds present within the exhaust medium (Schiraldi *et al.* 1999). Additionally, the proportion of glucose concentration to growth promoter concentration such as yeast extract (Krahe *et al.* 1996) or L-glutamate (Park & Lee 2000) was previously shown to determine the level of inhibition.

2-Propanol at 3.92 g l^{-1} or 4.02 g 1-propanol l^{-1} in the growth medium in combination with 10 g glucose l^{-1} inhibited cell growth. However, while no significant 1-propanol utilization was observed, 1.77 g 2-propanol l^{-1} (45% of initial concentration) was utilized within 28 d before the process halted (Figure 2A1). An accumulation of acetone up to 0.141 g l^{-1} in the medium suggested that 2-propanol was metabolized via the acetone metabolism pathway (Taylor *et al.* 1980). Evidence of 2-propanol metabolism via acetone by *S. solfataricus* has not been previously reported, despite its well-characterized zinc-dependent alcohol dehydrogenase (Ammendola *et al.* 1992, Can-

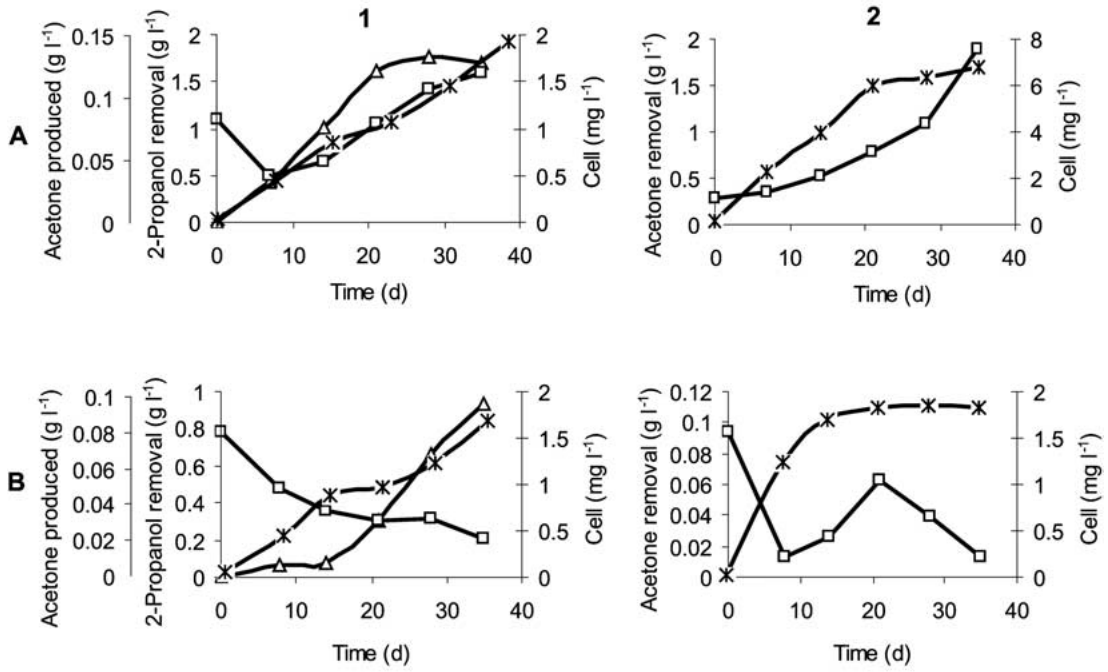

Fig. 2. Growth and degradation profiles of *S. solfataricus* with (A) and without (B) 10 g glucose l^{-1}. (1) Degradation of 3.92 g 2-propanol l^{-1} and acetone accumulation; (2) degradation of 3.95 g acetone l^{-1}. (□) Cell growth (expressed as protein content); (△) 2-propanol; (∗) acetone.

nio *et al.* 1996). These experimental data of the alcohol dehydrogenase activity characterization within the whole-cell system of *S. solfataricus* under various growth conditions, suggest that the 2-propanol metabolism pathway of the acidothermophilic archaeon, *S. solfataricus*, is similar to those present within mesophilic microorganisms.

In contrast to 1-propanol and 2-propanol, the presence of 3.95 g acetone l^{-1} growth medium, in combination with 10 g glucose l^{-1}, gave higher final biomass than when the culture was supplied by glucose alone, although it did not increase the growth rate (Figure 1). Up to 1.62 g acetone l^{-1} (41% of initial concentration) was utilized within 35 d of incubation (Figure 2A2), indicating that the presence of acetone did not interfere with glucose utilization by *S. solfataricus* (Giardina *et al.* 1986).

Completely different results were observed when the same amount of 2-propanol and acetone were added to the medium in the absence of glucose (Figure 2B). In spite of 0.93 g 2-propanol l^{-1} (24% of initial concentration) depletion and up to 0.085 g acetone l^{-1} accumulation detected in the medium within 35 d of observation, the amount of biomass (on a protein basis) decreased continuously (Figure 2B1). Therefore, not only 2-propanol failed to support *S. solfataricus* growth, but it is also inhibitory both in the presence or absence of glucose. Similarly, acetone utilization in the absence of glucose was also poor (Figure 2B2). The amount of biomass initially decreased, followed by a very low growth rate before the biomass decreased again, as acetone utilization halted at 0.11 g l^{-1} (2.5% of initial concentration) within 20 d.

In general, although *S. solfataricus* does not utilize 2-propanol as the sole source of carbon and energy, it co-metabolizes the compound via the acetone pathway. The substantial difference in 2-propanol and acetone utilization and their effect on biomass growth with or without glucose indicated that glucose was required to support growth. It is not clear if glucose also enhanced the 2-propanol metabolism (Figure 2). However, it has been postulated that alcohol dehydrogenase specific activity from *S. solfataricus* was higher in the early exponential phase when the growth medium was supplemented with glucose or aldehyde (Cannio *et al.* 1999). Incomplete degradation of 2-propanol and acetone was observed both with or without glucose due to several possibilities: (1) accumulation of toxic or inhibitory intermediates from 2-propanol, acetone, glucose and/or yeast extract, (2) lack of co-factor availability for continuation of enzymatic activity (for

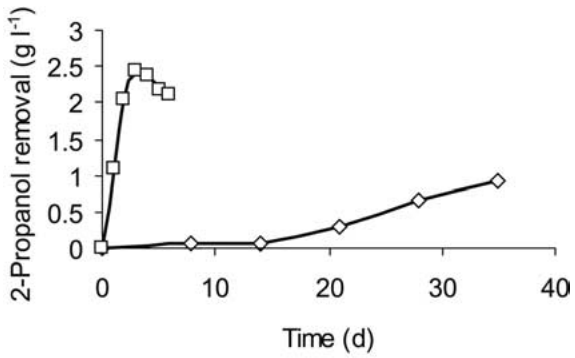

Fig. 3. 2-Propanol removal by *S. solfataricus* in suspended (◊) and immobilized culture (□) without glucose.

example NAD^+ for alcohol dehydrogenase), or (3) lack of other limiting factors such as sulphur or trace elements from the medium that may be involved in the metabolic process. These possibilities suggesting that suspended cultures were unfavourable for continuous metabolism of *S. solfataricus* and a system that is able to support continuous supply of nutrient as well as separate the cultures from toxic or inhibitory materials will give a better performance.

Effect of immobilized S. solfataricus *on solvent utilization*

Thermophilic archaea belonging to genus *Sulfolobus* produce an extracellular polysaccharide, a biofilm material, when grown on glucose (Nicolaus *et al.* 1993). Therefore, the aim to stimulate biofilm formation by immobilizing *S. solfataricus* within this study appeared feasible. Using Celite R-635 pellets as the support matrix, *S. solfataricus* was grown in ATCC 1304 medium supplemented with 3.92 g 2-propanol l^{-1} without additional glucose. Results showed that the immobilized culture of *S. solfataricus* was able to remove 2.5 g 2-propanol l^{-1} (64% of the initial concentration) within 3 d, while its suspended counterpart was only able to remove 0.93 g 2-propanol l^{-1} (25.5% of initial concentration) within 35 d (Figure 3). This means the 2-propanol removal rate by the immobilized *S. solfataricus* (0.035 g l^{-1} h^{-1}) was more than 25 fold higher than that of the freely suspended *S. solfataricus* (0.0012 g l^{-1} h^{-1}). Additionally, although 100% substrate removal was not achieved, the 2-propanol removal rate of immobilized *S. solfataricus* was still higher than that of suspended *S. solfataricus* supplemented with 10 g l^{-1} glucose (0.0026 g l^{-1} h^{-1}). The enhanced metabolic performance of the immobilized cultures appears to be promoted by biofilm formation (microscopy and electron microscopy – unpublished data), a process that favours slow growing microorganisms and provides protection from toxic or inhibitory materials and resistance to shock loading and antibiotics (Lee *et al.* 1994, Costerton *et al.* 1995, James *et al.* 1996). It has been reported previously that the formation of a biofilm by hyperthermophilic archaea, especially *Sulfolobus* sp., was a survival mechanism in response to environmental stresses (Weiss 1973, Nicolaus *et al.* 1993, Hartzell *et al.* 1999). Nevertheless, further experiments are required to determine the extent to which immobilization affects microbial metabolisms, for example how much it improves mineralization and whether it enhances metabolism through certain degradation pathways.

In conclusion, this work has documented the implementation of immobilized *S. solfataricus* for high temperature waste treatment, including the potential for the development of integrated chemical-biochemical VOC pollution abatement processes.

Acknowledgements

The authors thank the UK's Biotechnology and Biological Sciences Research Council (BBSRC) for financial support (97/E11124). PCW thanks the Engineering and Physical Sciences Research Council (EPSRC) for provision of an Advanced Research Fellowship (GR/A11311/01). We also would like to thank Prof D. A. Cowan for kindly providing the *S. solfataricus* isolate used in this work.

References

Ammendola S, Raia CA, Caruso C, Camardella L, Dauria S, Derosa M, Rossi M (1992) Thermostable NAD^+-dependent alcohol dehydrogenase from *Sulfolobus solfataricus* – gene and protein sequence determination and relationship to other alcohol dehydrogenases. *Biochemistry* **31**: 12514–12523.

Bustard MT, Meeyo V, Wright PC (2001) Biodegradation of high-concentration isopropanol vapour in a biofilter inoculated with a solvent-tolerant microbial consortium. *Trans IChemE* **79**: 129–135.

Bustard MT, Whiting S, Cowan DA, Wright PC (2002) Biodegradation of high-concentration isopropanol by a solvent-tolerant thermophile, *Bacillus pallidus*. *Extremophiles* **6**: 319–323.

Cannio R, Fiorentino G, Carpinelli P, Rossi M, Bartolucci S (1996) Cloning and overexpression in *Escherichia coli* of the genes encoding NAD-dependent alcohol dehydrogenase from two *Sulfolobus* species. *J. Bacteriol.* **178**: 301–305.

Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM (1995) Microbial biofilms. *Annu. Rev. Microbiol.* **49**: 711–745.

Derwent RG, Pearson JK (1997) Improving air quality in the United Kingdom – the solvents contribution. *Environ. Technol*. **18**: 1029–1039.

Fox P, Ketha S (1996) Anaerobic treatment of high-sulphate wastewater and substrate interactions with isopropanol. *J. Environ. Eng*. **122**: 989–994.

Giardina P, de Biasi MG, de Rosa M, Gambacorta A, Buonocore V (1986) Glucose dehydrogenase from the thermoacidophilic archaebacterium *Sulfolobus solfataricus*. *Biochem J.* **239**: 517–522.

Harris JW (1991) Hydrocarbon production handbook. *Hydrocarbon Proc*. **64**: 154.

Hartzell PL, Millstein J, LaPaglia C (1999) Biofilm formation in hyperthermophilic archaea. *Meth. Enzymol.* **310**: 335–349.

Hwang Y, Sakuma H, Tanaka T (1995) Denitrification with isopropanol as a carbon source in a biofilm system. *Water Sci. Technol.* **30**: 69–81.

James SG, Holmström C, Kjelleberg S (1996) Purification and characterization of a novel antibacterial protein from the marine bacterium D2. *Appl. Environ. Microbiol.* **62**: 2783–2788.

Krahe M, Antranikian G, Markl H (1996) Fermentation of thermophilic microorganisms. *FEMS Microbiol. Rev*. **18**: 271–285.

Lee CM, Lu CJ, Chuang MS (1994) Effect of immobilized cells on the biodegradation of chlorinated phenols. *Water Sci. Technol.* **30**, 87–90.

Nicolaus B, Manca MC, Romano I, Lama L (1993) Production of an exopolysaccharide from two thermophilic archaea belonging to the genus *Sulfolobus*. *FEMS Microbiol. Lett*. **109**: 203–206.

Park CB, Lee SB (2000) Effects of exogenous compatible solutes on growth of the hyperthermophilic archaeon *Sulfolobus solfataricus*. *J. Biosci. Bioeng*. **89**: 318–322.

Schiraldi C, Marulli F, Di Lernia I, Martino A, De Rosa M (1999) A microfiltration bioreactor to achieve high cell density in *Sulfolobus solfataricus* fermentation. *Extremophiles* **3**: 199–204.

Taylor DG, Trudgill PW, Cripps RE, Harris PR (1980) The microbial metabolism of acetone. *J. Gen. Microbiol.* **118**: 159–170.

Weiss BL (1973) Attachment of bacteria to sulphur in extreme environments. *J. Gen. Microbiol.* **77**: 501–507.