J Ind Microbiol Biotechnol (2006) 33:975–983
DOI 10.1007/s10295-006-0143-y

ORIGINAL PAPER

Bioconversion of isopropanol by a solvent tolerant *Sphingobacterium mizutae* strain

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Received: 31 January 2006 / Accepted: 9 May 2006 / Published online: 7 June 2006

Abstract The bioconversion of high concentration isopropanol (2-propanol, IPA) was investigated by a solvent tolerant strain of bacteria, which was identified as *Sphingobacterium mizutae* ST2 by partial 16S rDNA gene sequencing. This strain of bacteria exhibited the ability to utilise high concentration isopropanol as the sole carbon source, with mineralization occurring via an acetone intermediate into central metabolism. The biodegradative performance of this strain for IPA was examined over a 2–38 g l^{-1} concentration range, using specific growth rate (μ) and conversion rate analysis. Maximum specific growth rates (μ_{max}) of 0.0045 h^{-1} were routinely obtainable on IPA. In addition, the highest specific IPA degradation rate was obtained at a concentration of 7.5 g l^{-1} with a corresponding value of 0.045 g IPA g $cells^{-1}$ h^{-1}. While the highest acetone yield reached its maximum value of 0.940 g acetone g IPA^{-1} at 7.5 g IPA l^{-1}. This is the first report on bioconversion of isopropanol at such high concentration by this solvent tolerant strain of *S. mizutae* and may allow its application in novel biocatalytic processes for effective biological conversion in two-phase solvent systems.

Keywords Solvent tolerant · Biodegradation · *Sphingobacterium mizutae* · Isopropanol (IPA) · Acetone · Specific growth rate · Inhibition kinetics

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Introduction

The world-wide production of 2-Propanol, or isopropyl alcohol (IPA), exceeds 1.8 million metric tonnes per annum [38]. It is used particularly as a solvent in quick-drying oils, inks, cosmetics, anti-freeze compositions, and as a cheap replacement for ethanol [7]. Since it is widely used industrially, this has resulted in an increased production of organic solvent waste streams, where it can be shown that IPA represents the largest volume solvent release compound in the UK, at nearly 7% of the total emission [10].

Biological methods offer an attractive, cost effective method of converting solvent wastes [8], where biological processes would be considered to be substantially more attractive to industry if bacteria were able to operate in high concentrations of solvent. The reason being that smaller and cheaper waste treatment facilities would be possible, thus widening the appeal of pollution abatement strategies by biological techniques. In order for the biodegradation process to take place, good physical contact between the compound and the microorganism is essential, but in fact numerous problems appear in the application of biological systems to solvent treatment [4], due to the toxic effects of such organic solvents on the bacteria. A direct

relationship exists between the accumulation and partitioning of a particular solvent in the cell membrane and the effect on cellular function [35]. These effects include: (a) alteration in the composition and integrity of both the outer cell and cytoplasmic membrane [22], hence changing the permeability of the membrane and subsequently affecting its function, which often results in lysis of the microorganism [9]; (b) decreasing the proton motive force, and thus ATP synthesis by leakage of protons and ions out of the cell; (c) increasing the fluidity of the membrane; (d) inhibiting functions of membrane proteins via transfer of proteins extracellularly.

Such harmful effects of organic compounds on certain strains of bacteria have been studied, for example the effect of butanol on *Clostridium acetobutylicum* [6], where it was found that at a high butanol concentration of approximately 18.5 g l^{-1} resulted in inhibition of ATP levels, and disruption of the membrane fluidity. Similarly, the combined effect of temperature and ethanol was characterized for *Clostridium thermocellum*, and its mutant, at up to 20 g l^{-1}. This bacterium was not able to utilize ethanol as its carbon source, but exhibited increased membrane fluidity as a response to the presence of high ethanol concentrations [16]. A similar effect has been reported during growth of *Escherichia coli* in a medium containing 31.6 g l^{-1} ethanol [11], which caused a change in membrane lipid composition. In addition to *E. coli*, similar changes have been characterized with *Bacillus cereus* during growth on ethanol or 1-propanol medium, at concentrations of 9.21 and 4.81 g l^{-1} respectively [24].

Several mechanisms have been adopted by microorganisms in order to overcome the effect of the accumulation of organic solvents, including increasing the degree of saturation of the bilayer lipids in the membrane, for example. This behaviour reduces the fluidity of this membrane, which can be achieved by cellular synthesis of the lipids, or by *cis/trans* isomerization of the unsaturated fatty acids, thus preventing the leakage of protons, ions, and proteins from the cell [15]. This process changes the configuration of the double bond of the fatty acid from the *cis* configuration (condition of high fluidity of the membrane due to their bent structure) into the long, extended *trans* configuration. This process allows the membrane unsaturated:saturated fatty acid ratio to increase, thus improving the integrity of that membrane [19].

Another physiological mechanism adopted by microorganisms to decrease the amount of toxic solvents in the cell, is the extracellular transportation of the solvent molecules out of the cytoplasm. This occurs through the outer membrane, via efflux transporters consisting of consecutive efflux pumps [25]. The importance of such a system arises from the suggestion that increasing tolerance towards organic solvents in microorganisms is often not as a result of the solvent metabolism, but rather the efflux pump system.

In recent years, several different strains of solvent tolerant bacteria have been isolated. For example, a *Flavobacterium* sp. was able to tolerate benzene at concentrations up to 43.83 g l^{-1} [31], while a *Bacillus* sp. demonstrated survival in both benzene and toluene at concentrations up to 43.83 and 86.60 g l^{-1} respectively [30]. Furthermore, a *Rhodococcus* sp. was shown to tolerate a lower concentration of benzene up to 17.53 g l^{-1} [31], and exhibition of ability to survive a very high concentration of toluene up to 433 g l^{-1} has been observed in *Pseudomonas* sp. [21]. It has also been reported that *Pseudomonas putida* can degrade toluene in liquid culture at the concentration range of 0.0092 up to 0.0138 g l^{-1} by either of two ways: oxidation of methyl group to alcohol, or the attack of the aromatic nucleus by the oxygenase enzyme [39].

Previously, we have shown that a mixed consortium, isolated and enriched from oil to contaminated soil, is capable of degrading up to 7.9 g l^{-1} IPA as its sole carbon source [7]. One species isolated from this consortium is investigated here in terms of its ability to metabolize high concentrations of the secondary alcohol, IPA [7]. In this study, we describe the mineralization of IPA up to 38 g l^{-1} by this strain for the first time. These conditions are five times higher than previous reports of IPA-degrading bacteria, and at least one order of magnitude greater than other microbial IPA/acetone aerobic degradation reports in the literature [20].

Methods and materials

Isolation, enrichment, and maintenance of bacterial strains

Bacteria were isolated from hydrocarbon enriched cultures as described by Bustard et al. [7]. Samples of an oil/soil mixture (0.2 g) obtained from a waste sump at Heriot-Watt University, UK, were placed in a 250-ml Erlenmeyer flasks containing 7.3 g $^{-1}$ decane in 100 ml of mineral salt medium (MSM) containing the following constituents per litre of deionized water, 3 g $NaHCO^{3}$, 1 g NH_4HCO_3, 0.2 g K_2HPO_4, 102.5 mg $MgSO_4.7H_2O$, 36.75 mg $CaCl_2.2$-H_2O, 10 mg $FeSO_4$, 1 ml trace elements solution) at pH 6, the formulation of MSM was adapted from Angelidaki et al. [3].

Enrichment was carried out with 7.9 g l^{-1} 2-propanol as carbon source, within 100 ml mineral salt medium (MSM), the liquid cultures were allowed to grow at 20°C on an KS250 orbital shaker (IKA Werke, Germany) at 150 rpm. Once bacterial growth was observed, and 2-propanol utilized (approximately 7 days), liquid samples (5 ml) were extracted and used to inoculate new flasks under the same conditions as used previously. This cycle was repeated several times. Pure bacterial cultures were obtained by appropriate dilution of the final cell suspension, and were spread on nutrient agar plates. The pure strains obtained were either stored at 4°C for the short term, and transferred onto new agar plates every 6 weeks, or stored in 60% glycerol stocks at –80°C for the long term, until required.

Cultivation of solvent utilizing microorganisms

Single colony forming units were selected from the nutrient agar plates, inoculated with this 2-propanol-utilizing culture. Subsequently, these single strains were inoculated in MSM with 7.9 g l^{-1} 2-propanol as carbon source. They were cultivated in an incubator/shaker at 150 rpm and 20°C. The growth was monitored periodically, by observing culture densities, and measuring cell concentration. Cultures were also examined under a CH–2 light microscope (Olympus America Inc., US) to ensure single strain purity. Periodically, nutrient agar spread plates were generated for additional confirmation of strain purity.

Isolation and analysis of 16S rDNA

Genomic DNA of the strain was extracted using the Prepman purification kit and stored on ice until use. The 16S ribosomal DNA gene was amplified using universal eubacterial primers [28], visualised by electrophoresis on a 1% agarose gel, with the resulting 500 bp fragment purified by spin column centrifugation and resuspended in sterile distilled water. The purified DNA product was then sequenced using the dideoxy chain terminator method as described by Sanger et al. [33]. Finally, the partial sequence of the 16S rDNA gene was compared with nucleic acid sequence databases (Microseq and BLAST) [32].

Determination of phenotypic characteristics

An API 20NE test kit (bioMerieux, Marcyl'Etoile, France) was also used for further identification, by incubating the bacteria at 30°C and examining test strips after 24 and 48 h, according to the manufacturer's instructions [2]. This allowed for additional determination of physiological and biochemical characteristics of the strain.

2-propanol (IPA) batch degradation experiments

Bacterial inocula were prepared by sub-culturing into MSM with 7.9 g l^{-1} 2-propanol, incubated at 20°C in a rotary shaker at 150 rpm, samples were taken every 24 h to measure bacteria growth, once the growth had reached the end of exponential growth phase, cells were taken to initiate biodegradation studies as follows: 25 ml Erlenmeyer flasks were set up, containing six different concentrations of IPA in duplicate flasks (concentration ranges between 2 and 79 g l^{-1}). Each flask contained 10 ml of a MSM to which 1 ml of an inoculum containing 1.4×10^{9} cells was added, which was equivalent to 0.240 g dry cell weight (DCW) l^{-1}. In order to correct for volatilization/evaporation, multiple control samples were also set up in parallel using the six corresponding concentrations of IPA in addition to 10 ml MSM, on a cell-free basis. Rates of acetone evaporation were also calculated. The flasks were stoppered with foam bungs and placed on an IKA KS250 orbital shaker at 150 rpm at 20°C for the duration of the experiment. Well-mixed samples were taken from each flask every 24 h for solvent concentration and cell growth analysis.

Microbial growth measurements

Samples were withdrawn periodically for analysis. Cell growth was monitored spectrophotometrically by absorbance measurement at a wavelength of 450 nm (λ_{450}) using a DR/2000 spectrophotometer (Hach, Germany), and the corresponding total cell number determined using a haemocytometer slide 0.1 mm × 0.0025 mm (Superior, Germany) and an Olympus CH-2 microscope. A calibration curve for optical density (OD_{450}) and cell number (CN) ml^{-1} was then established.

Dry cell weight (DCW) was determined by measurement of cell concentration as follows; 50 ml culture broth was harvested by centrifugation at 7,000 rpm for 10 min, on a Mark IV refrigerated centrifuge (Baird and Tatlock, England), the pellet was washed extensively with distilled water and then scraped into a preweighed aluminium pan, followed by drying in an oven (Gallenkamp, UK) at 80°C until constant weight and cooled in a desiccator prior to re-weighing. One OD_{450} unit of was found to be equivalent to 1.450 g l^{-1} *Sphingobacterium mizutae* ST2.

Solvent concentration determination

A 1 μl sample was extracted by syringe from a well-mixed uniformly suspended culture from each flask and analysed with a GC-17A Gas Chromatograph (Shimadzu, Japan). This was equipped with a Flame Ionisation Detector (FID) in order to determine the concentration of IPA and its metabolite, acetone. The GC was equipped with a Carbowax BP20 column (length = 15 m, 1 μm film) (Burke analytical, UK). The flow rate of the He carrier gas was 10 ml min^{-1}, the temperature of the FID system was 300°C, whereas that of the liquid injection point was 250°C, and the oven temperature was 70°C. The run time was 2 min.

Experimental bacteria depository

The *Sphingobacterium mizutae* ST2 used in this study has been deposited in the American Type Collection Culture (ATCC) under accession number ATCC BAA-327.

Nucleotide sequence accession numbers

The partial 16S rDNA gene sequences obtained in this study for *S. mizutae* ST2 has been deposited in the GenBank database under accession number AF361550.

Calculations

The kinetics of growth and substrate degradation in batch culture are described by simple differential equations [14, 34].

Cell growth is described by

$$\frac{dX}{dt} = \mu X, \quad X = X_0 \quad \text{at} t = 0, \tag{1}$$

where X is cell concentration, t is cultivation time, and μ, specific growth rate.

A semi-logarithmic plot of cell concentration versus time was prepared for each experimental run, and the specific growth rate (μ) for a range of initial 2-propanol concentrations was determined from the slope of the linear portion (the exponential cellular growth phase) by applying non-linear regression.

The specific degradation rate of the substrate, q, was determined as follows:

$$-\frac{dS}{dt} = qX, \tag{2}$$

where S is substrate concentration, and q, specific degradation rate.

Integrating the above equations yields:

$$X = X_0 \exp(\mu t), \tag{3}$$

$$[S]_0 - [S] = q\frac{1}{\mu}(X - X_0), \tag{4}$$

where X_0 is initial cell concentration, and $[S]_0$, initial substrate concentration.

Hence a plot of ($[S]_0$–$[S]$) versus (X–X_0)/μ should give a straight line of slope q. Only data confined to the exponential growth phase was used for analysis.

The volumetric degradation rate, r, is defined as follows:

$$r = \frac{[S]_0 - [S]_{t_c}}{t_c}, \tag{5}$$

where t_c is total cell culture time of each batch cycle, and $[S]_{t_c}$, final substrate concentration during the exponential growth phase of each batch cycle.

Results

Taxonomic characterization of *S. mizutae*

A Gram-negative strain of bacteria growing in mineral salt medium (MSM) with isopropanol as a sole carbon and energy source was identified as *S. mizutae* ST2. The phenotypic characteristics of the strain were determined with the API 20NE test, and were compared with the reference database in the API test manual, and available reference data [2]. Test results are summarized in Table 1. The strain was able to assimilate glucose, arabinose, mannose, malate, citrate, mannitol, *N*-acetyl-glucosamine, maltose and adipate as a sole carbon source. However, comparison of the results obtained for *S. mizutae* with other published information was difficult, since little reference data for *S. mizutae* is currently available.

The strain was studied further by 16S rDNA gene characterization. The sequence obtained was compared to those in the Microseq database. Although it was not clear if a genus level had been obtained by Microseq database, which identified the best match to *Sphingobacterium thalpophilum* with 89.81% similarity, a further search was carried out using EMBL database, and the best match from a FASTA search [27] at 94.10% similarity is *S. mizutae*. On the other hand, the phylogenetic tree obtained (data not shown) indicated that the Gram-negative bacterium *Flavo-*

Table 1 Phenotypic characterisation for the identification *S. mizutae* ST2

Characteristic/substrate utilization	*S. mizutae*
Nitrate reduction	+
Indole production	–
Acidification	–
Arginine dihydrolase	–
Urease	+
Hydrolysis (*β*-glucosidase)	–
Hydrolysis (protease)	+
β-galactosidase	–
Assimilation of:	
Glucose	+
Arabinose	+
Mannose	+
Mannitol	+
N-acetyl-glucosamine	+
Maltose	+
Gluconate	–
Caprate	–
Adipate	+
Malate	+
Citrate	+
Phenyl-acetate	–

bacterium sp., is the most closely related genus to *Sphingobacterium* sp. It has been reported in the literature, through taxonomic studies, that *S. mizutae* should continue to be retained to the genus *F. mizutae*, due to their similar phenotypic characteristics [17, 36]. Table 3 summarizes the phenotypic characteristics of selected *Flavobacterium* and *Sphingobacterium* strains as studied by Takeuchi and Yokota [36].

Cell growth on 2-propanol (IPA)

S. mizutae ST2, demonstrates the ability to grow on IPA with no further C supplementation in the range of 2–38 g l^{-1} as shown in Fig. 1a and b. By increasing 2-propanol concentrations, the lag period for *S. mizutae ST2* increased significantly. The lag time was estimated to be 24 h for an initial IPA concentration of 2 g l^{-1} (Fig. 1a), while increasing the initial concentration to 15 and 38 g l^{-1} caused an increased lag time to 69 and 170 h, respectively (Fig. 1b). Furthermore, increasing the initial IPA feed concentration to 79 g l^{-1} caused complete inhibition of bacterial growth for *S. mizutae* ST2. It is clear from the cell growth patterns for *S. mizutae* ST2 that increasing the IPA concentrations causes an inhibition in bacterial growth, and this was demonstrated by measuring the specific growth rate (μ) at different IPA concentration ranges from 2 to 25 g l^{-1}. The specific growth rate data are plotted versus initial 2-propanol concentration as shown in Fig. 2. It is clear that specific growth rate is reduced at higher 2-propanol

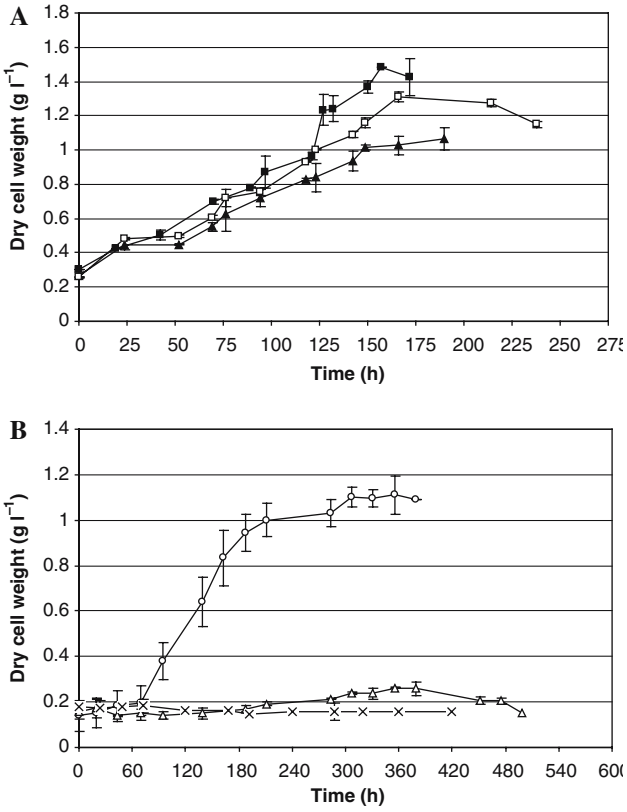

Fig. 1 **a** Growth of *S. mizutae* ST2 based on dry cell weight at an initial IPA concentration of 2 (*triangle*), 3.9 (*open square*), and 7.9 g l^{-1} (*filled square*) in batch cultures. **b** Growth of *S. mizutae* ST2 based on dry cell weight at an initial IPA concentration of 15 (*circle*), 38 g l^{-1} (*triangle*), and 79 g l^{-1} (*cross*) in batch cultures

concentrations. After increasing the initial IPA concentration from 7.9 g l^{-1} a decrease in the maximum growth rate to 0.002 h^{-1} was observed at initial 2-propanol concentrations up to 25 g l^{-1}, thereby indicating possible substrate inhibition at higher solvent concentrations.

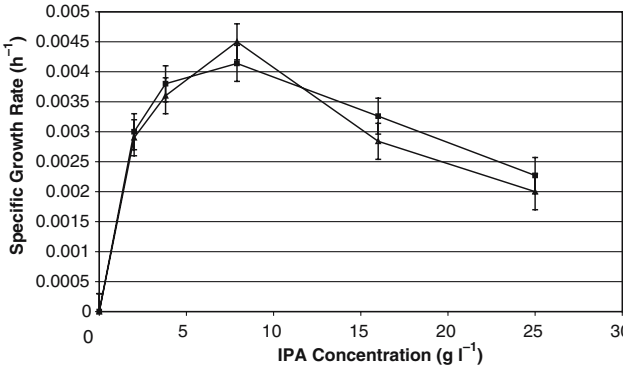

Fig. 2 Specific growth rate based on experimental data (*triangle*), and Edwards model correlation (*square*) for *S. mizutae* ST2 on 2-propanol

Negligible growth was observed at 38 g l^{-1} IPA substrate. The specific growth rate (μ) versus 2-propanol concentration (S) data was used to determine the kinetic parameters of bacterial growth in batch cultures containing 2-propanol (IPA) at initial concentration of 2–25 g l^{-1}. Some of the models that were tested include: Haldane model [37], Aiba et al. [1], Edwards [12], and Levenspiel [27]. Among these, the best representative model to express the kinetic behaviour of the bacterium over the 2-propanol concentration range studied (2–25 g l^{-1}) was the Aiba and Edwards model displaying the following kinetic parameters: saturation constant (K_s=3.31 g l^{-1}), inhibition concentration (K_i=20.82 g l^{-1}) and R^2=0.95 (Fig. 2). The Aiba Edwards model could be considered a reasonable kinetic model to describe the inhibition trend by IPA for *S. mizutae*, where at low IPA concentrations of up to 3.9 g l^{-1} good agreement between the experimental and predicted data was evident. However, at higher IPA concentrations (above 10 g l^{-1}) there was a noticeable deviation from the model indicating a high concentration effect not taken into account by the Edwards model.

Batch bioconversion of 2-propanol

The ability of *S. mizutae* ST2 to convert IPA was examined by studying the volatilisation-corrected biodegradation rate of IPA at initial concentrations ranging from 2 to 38 g l^{-1}. It was observed that acetone is produced as the main intermediate during IPA metabolism. Figure 3a and b show the time-dependent concentration change in IPA and acetone production for *S. mizutae* ST2 over the stated range. The results show that IPA was not fully converted at concentrations of 15.8 and 38 g l^{-1}, with a total degradation of 83 and 49% evident for these respective concentrations. In order to compare the bacterial growth performance at different 2-propanol concentrations, the specific degradation rates, volumetric degradation rates, and acetone yield coefficient were calculated, results shown in Table 2 indicate that increasing the initial IPA concentration results in an increase in the specific degradation rate and volumetric degradation rate up to a certain inhibitory IPA concentration, where a trend change was observed. For example, increasing the IPA concentration from 2 to 7.5 g l^{-1} resulted in an increase in the specific degradation rate from 0.014 to 0.045 g IPA g $cells^{-1}$ h^{-1}, with a corresponding increase in volumetric degradation rate from 0.019 to 0.038 g l^{-1} h^{-1} (Fig. 3a). However, a transition point was evident where a further increase in initial IPA concentration inhibited the degradation rates, as is clearly demonstrated via specific degradation values, where

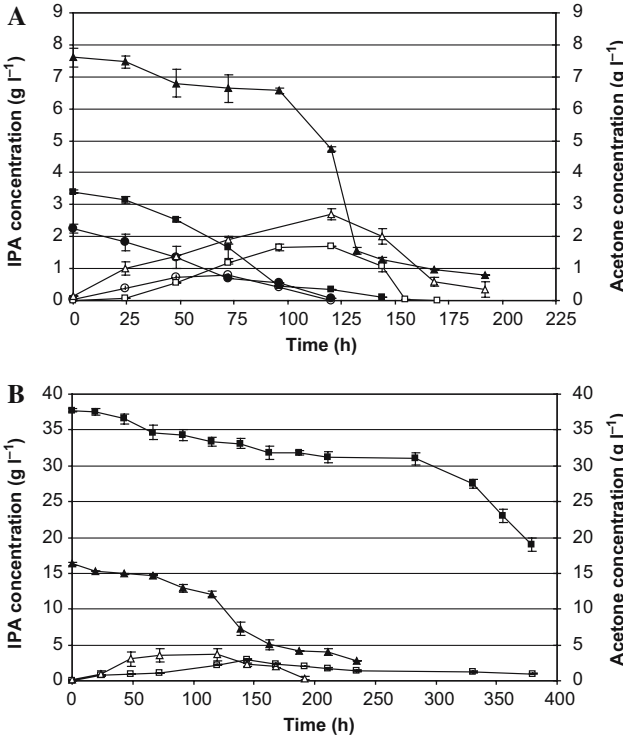

Fig. 3 **a** The bioconversion of 2 (*filled circle*), 3.9 (*filled square*), and 7.5 (*filled triangle*) g l^{-1} 2-propanol, and the subsequent acetone production during the process (corresponding *open symbols*). **b** The biodegradation of 15 (*filled triangle*), and 38 (*filled square*) g l^{-1} 2-propanol, and the subsequent acetone production during the process (corresponding *open symbols*)

increasing the IPA concentration beyond 7.5 g l^{-1} resulted in significant decrease where values reached 0.025 and 0.010 g IPA g $cells^{-1}$ h^{-1} at IPA concentrations of 15.8 and 38 g l^{-1}, respectively (Fig. 3b). This transition point was also clear in the volumetric degradation rate data. For instance, the volumetric degradation value reaches its maximum at 15.8 g l^{-1} IPA, with a value of 0.057 g l^{-1} h^{-1}, and increasing the IPA concentration to 38 g l^{-1} inhibited the volumetric degradation rate to 0.024 g l^{-1} h^{-1} (Table 2).

It was found that acetone was produced as an intermediate in the aerobic bioconversion of 2-propanol and

Table 2 Specific IPA degradation rate (q), volumetric IPA degradation rate (r), and acetone yield coefficient (Y) for *S. mizutae* ST2

Initial IPA concentration (g l^{-1})	q (g IPA g $cells^{-1}$ h^{-1})	r (g l^{-1} h^{-1})	Y (g acetone g IPA $^{-1}$)
2	0.014	0.019	0.490
3.9	0.019	0.021	0.555
7.5	0.045	0.038	0.940
15.8	0.025	0.057	0.832
38	0.010	0.024	0.450

its concentration in the medium was measured throughout experiments. A maximum yield coefficient of 0.940 g acetone g IPA^{-1} was obtained at an initial IPA concentration of 7.5 g l^{-1}, followed by a value of 0.832 g acetone g IPA^{-1} at 15.8 g l^{-1}. In contrast, the minimum yield of acetone was obtained at 38 g l^{-1} with a value of 0.450 g acetone g IPA^{-1}. It may be concluded from the results that increasing the IPA feed concentration towards 38 g l^{-1} caused a decrease in the degradation rates and yield values, which is likely to be the result of substrate inhibition of microbial growth at high 2-propanol concentration.

Discussion

The scope of this study was to study the ability of a solvent tolerant bacterial strain named as ST2 to utilize 2-propanol (IPA) as its carbon and energy source under aerobic conditions. This bacterium was one of the dominant strains from a soil–oil sump isolated previously from a consortium used by Bustard et al. [7] in the study of 1-propanol and 2-propanol degradation. The dominant strain has been characterized with the standard API 20NE identification system, and further identified as *S. mizutae* ST2 by 16S rDNA gene sequencing. These results for strain ST2 displayed a 94.10% similarity with *Sphingobacterium mizutae*, which could be retained to *Flavobacterium mizutae*, due to their similar phenotypic characteristics as suggested by Holmes et al. [17]. In a review by Holmes [18] the *Flavobacterium* genus was divided into four groups, one of those groups included *F. mizutae*, *F. multivorum*, *F. spiritivorum*, *F. thalpophilum*, and *F. yabuuchiae*. Most of these organisms were shown to possess novel sphingolipids in their cell walls hence the name *Sphingobacterium* was proposed [41]. However, according to a study conducted later by Bernardet et al. [5], the *Flavobacterium* family was reclassified and the genus *Sphingobacterium* (with *S. mizutae*, *S. heparinum*, *S. spiritivorum*, and *S. thalpophilum*) was not included.

Both Gram-negative and Gram-positive bacteria are able to tolerate different organic solvents, although it has been demonstrated by Isken and de Bont [8] that Gram-negative bacteria are more likely to show solvent tolerance than Gram-positive, due to the presence of an additional outer membrane. The biodegradation study carried out here at a range of initial 2-propanol (IPA) concentrations shows the solvent-tolerant Gram-negative bacterium *S. mizutae* ST2 and its ability to convert high concentration 2-propanol (to 38 g l^{-1}), which may be as a result of a gradual adaptation the organic solvent.

Sphingobacterium sp. has been reported to biodegrade certain organic compounds such as: benzo[a]pyrene at concentrations of up to 0.10 g l^{-1} [23], and oleic acid at concentrations of up to 8.70 g l^{-1} [26], for example. *Flavobacterium* can mineralize halogenated phenols such as pentachlorophenol completely into CO_2, Cl^-, and H_2O [29], by dechlorination and hydroxylation steps through the PCP-4-monooxygenase enzyme, which also catalyzes several reactions of chlorinated phenol compounds [40].

Literature is rich in studies that have been carried out showing the ability of *Sphingobacterium* sp. to degrade various compounds at a range of concentrations,

Table 3 Biochemical characteristics of the strains of the genera *Sphingobacterium* (Takeuchi and Yokota [36])

Characteristic/substrate	*F. thalpophilum*	*S. mizutae*	*F. spiritivorum*	*F. yabuuchiae*
Nitrate reduction	+	–	–	–
Nitrite reduction	–	+	–	–
Urease	+	+	+	+
Dnase	–	+	+	+
Assimilation of:				
Mannitol	–	–	+	+
N–acetyl-glcosamine	+	–	+	–
Sorbitol	–	–	–	–
D-Arbitol	–	–	–	–
Glycerol	–	–	–	–
L-Fucose	+	–	+	+
Melibiose	+	+	+	+
Xylitol	–	–	–	–
L-Sorbose	–	–	–	–
5-keto-gluconate	–	–	–	
Enzyme activity:				
Lipase	–	+	–	+
β-Glucuronidase	+	–	+	+
Trypsin	+	+	+	+

but no studies previously demonstrated the biodegradation of high concentration 2-propanol by this strain of bacteria under aerobic conditions. In our studies, the conversion capacities of the strain was studied in the presence of up to 38 g l^{-1} IPA, with highest specific degradation rate of 0.045 g IPA g $cells^{-1}$ h^{-1} obtained at an IPA concentration of 7.5 g l^{-1}.

In order to understand the relationship between higher initial 2-propanol concentration and the specific growth rate, several kinetic models were tested to fit the growth rate at different substrate concentrations, where the objective was to find a representative kinetic model to describe the substrate inhibition quantitatively. At low IPA concentration (to 3.9 g l^{-1}) good agreement between the experimental and Aiba Edwards model-predicted data for *S. mizutae* ST2 occurred but at higher substrate concentration there was a noticeable deviation from the model. Therefore, this model could form the basis for future work to formulate a more representative model to describe the growth rate inhibition by 2-propanol at high concentration.

In the literature, several articles exist comparing a range of kinetic models to express growth kinetics of microorganisms inhibited by substrate. Most models have been formulated to describe the substrate inhibition phenomenon at low substrate concentration in comparison to the IPA concentration employed here. Nevertheless, there are no references evaluating kinetic models for substrate inhibition during biodegradation of high concentration 2-propanol by these solvent-tolerant bacteria. Thus the deviation observed between the experimental and correlation data could be due to the nature of the substrate and the high concentrations range tested here.

Although several mechanisms have been suggested for the oxidation of alcohols and ketones, it is still unknown whether the IPA degradation pathways of *S. mizutae* are due to the function of a broad specificity oxidoreductase enzyme, which may have alternative binding sites for oxidation and reduction reactions, or due to the catalytic activity of two enzymes. For example an alcohol dehydrogenase enzyme, oxidizing the hydroxyl group, into a keto group, in addition to a second possible enzyme, which may be acetone monooxygenase.

In our study, it may be hypothesised that *S. mizutae* ST2 shows an ability to aerobically convert IPA to acetone at high concentration. In fact, a variety of bacteria are capable of utilizing acetone itself as a growth-supporting substrate. Studies involving these bacteria have provided evidence leading to several proposed pathways for bacterial acetone metabolism and are discussed elsewhere [13].

Conclusion

This study demonstrates for the first time, the ability of a single strain of bacteria, identified as *S. mizutae* ST2, to tolerate, convert and grow on high concentrations of 2-propanol under aerobic conditions in free suspension, with a μ_{max} of up to 0.0045 h^{-1}. In this study, degradation rates were strongly influenced by the initial 2-propanol concentrations. The highest specific degradation rate obtained (q) was 0.045 g IPA g $cells^{-1}$ h^{-1} for *S. mizutae* with 7.5 g l^{-1} 2-propanol.

This strain of solvent-tolerant bacteria shows strong promise for the future development of cost effective biological solvent conversion processes or biocatalysis. Ability to tolerate and mineralize high concentrations of solvent widens the opportunities for biological treatment into areas traditionally catered for by chemical and physical techniques, which do not involve a pre-treatment step for effluents to render them suitable for 'normal' biological conditions.

Acknowledgments B.T.M acknowledges Heriot-Watt University for the provision of a scholarship. The UK's Biotechnology and Biological Sciences Research Council (BBSRC) is also thanked for provision of a research grant (Ref 97/E11124). P.C.W thanks the EPSRC for an Advanced Research Fellowship (GR/A11311/01), and M.T.B thanks the Royal Academy of Engineering's Research Fellowship Scheme.

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