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# Germanium toxicity in selected bacterial and yeast strains

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## SUMMARY

The toxicity of germanium dioxide ($GeO_2$) to 21 bacterial and 13 yeast strains was investigated in liquid broth medium to obtain information on strains tolerant to high (1 to 2 mg/ml) $GeO_2$ concentrations. *Arthrobacter* sp. NRC 32005, *Enterobacter aerogenes* NRC 2926, *Klebsiella aerogenes* NCTC 418 and *Pseudomonas putida* NRC 5019 were tolerant to 1 mg/ml $GeO_2$. *Bacillus sp.* RC607 was able to grow in the presence of 2 mg/ml $GeO_2$ at pH 10 in broth culture. The yeasts *Candida guilliermondii, Candida shehatae*, and *Pachysolen tannophilus* were the most sensitive to $GeO_2$ as evidenced by their diminished growth rates at a $GeO_2$ concentration as low as 0.1 mg/ml. None of the yeast strains tested exhibited growth in the presence of 1 mg/ml $GeO_2$. The high pH of the medium containing germanium may be partially responsible for the growth inhibition of the yeast cultures. Select bacterial cultures previously exposed to 1 mg/ml $GeO_2$ could tolerate and grow better at 2 mg/ml $GeO_2$, suggesting the existence of very efficient adaptive mechanisms. The pH of the medium could modulate $GeO_2$ tolerance and this effect was found to be strain-dependent.

## INTRODUCTION

Germanium (Ge) is a semi-precious, biologically non-essential metal with considerable potential for application in the fields of electronics, computers and medicine. Germanium normally occurs at a crustal abundance of 1.5 mg/kg [3]. It can also be found in waste products of the coke and coal industries [5]. Industrially, most of the germanium (approximately 90%), is used in the manufacture of semiconductor devices [3]. Germanium is used also as a component in special optical glass and infrared lenses; or in alloys with aluminum, aluminum–magnesium and tin to increase strength and hardness. In addition, germanium can act as a low temperature catalyst for hydrogenation of coal [3].

Recently, there have been reports that germanium may possess biological activities. Mochizuki and Kada [6] reported that an organogermanium compound, carboxyethylgermanium sesquioxide

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(Ge-132) exerted an antimutagenic effect on γ-ray-induced mutations in an *Escherichia coli* strain. Kada et al. [4] found a similar antimutagenic effect with germanium dioxide ($GeO_2$) on frameshift reverse mutations induced by 3-amino-1-methyl-5H-pyrido [4,3-b] indole in *Salmonella typhimurium* strains. It has also been reported that organogermanium possesses antitumor activity in mouse tumors, an effect attributable to Ge inducing the production of interferon [2].

There is limited information on the bioaccumulation of Ge by microorganisms. This information is necessary if organisms are to be used in Ge recovery processes. Yanagimoto et al. [8] reported that Ge uptake by several algae could be significantly enhanced when the pH of the medium was adjusted to more alkaline values in which the strains could not grow. Klapcinska and Chmielowski [5] and Chmielowski and Klapcinska [1] reported on the binding of Ge to *Pseudomonas putida* cells that were grown in a medium containing $GeO_2$ and catechol or acetate. Electron microscopy showed the Ge was bound within the Gram-negative cell envelope. In addition, a number of small, dense Ge deposits were observed in the cytoplasm of cells previously grown in the presence of both $GeO_2$ and catechol. Apparently, catechol aided in the intracellular bioaccumulation of $GeO_2$. The uptake of $GeO_2$ by *P. putida* cells was also found to occur in a biphasic manner [1].

It is not known if Ge accumulation is a common phenomenon among microorganisms. Moreover, little is known about Ge toxicity in bacteria or yeasts. The present study identified several bacterial and yeast strains which were tolerant or sensitive to $GeO_2$. Germanium-sensitive strains may be potentially useful because the sensitivity could result from rapid intracellular accumulation of the metal. Tolerant strains may possess mechanism(s) that exclude Ge, thereby preventing its accumulation inside the cell, where it may exert toxic effects.

## MATERIALS AND METHODS

### *Bacterial and yeast strains*

Twenty-one bacterial strains representing 8 different genera, and 13 yeast strains representing 8 genera were studied for their tolerance to $GeO_2$. The organisms were obtained from a variety of sources, including the National Research Council Canada (NRCC) through the courtesy of R.K. Latta; American Type Culture Collection (ATCC), National Collection of Yeast Cultures (NCYC), National Collection of Type Cultures (NCTC), and the National Collection of Marine Bacteria (NCIMB). Bacterial strains were also obtained from the following sources: *Pseudomonas fluorescens* strain FRI from G.G. Geesey, California State University; *P. putida* strain P.S. from P.J. Sadler, Birkbeck College, University of London, U.K.; *P. stutzeri* strain AG 259 from K. Hardy, Biogen S.A. Switzerland; *P. ambigua* from the Department of Agricultural Chemistry, Gifu University; and *Bacillus* sp. RC607 from Brandeis University. The yeasts *Saccharomyces cerevisiae* X2180-1B, *Debaryomyces hansenii* NCYC 459 and *Rhodotorula rubra* NS-76-138 were from G.M. Gadd, University of Dundee, Dundee, U.K. In addition, some of our own strains from the University of Guelph were screened. A list of the organisms is provided in Table 1. Many of these organisms were selected for this study because they were pseudomonads, or were tolerant to metals other than Ge.

Bacteria were maintained at 4°C on nutrient agar slants (Difco). Yeasts were maintained at 4°C on agar slants containing (% w/v): yeast extract, 1.0; peptone, 2.0; dextrose, 2.0; and agar, 2.0. For long-term storage, cultures were grown to late log phase and stored in 10% sterile glycerol at −20°C.

### *Chemicals*

Ultrapure $GeO_2$ was purchased from Johnson Matthey Inc., Toronto. A stock solution of 10 mg/

Table 1

Growth responses of organisms in the presence of germanium dioxide ($GeO_2$) at 28°C

| Organisms | $GeO_2$ Concentration (mg/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | 0.1 | | 0.5 | | 1.0 | | 2.0 | |
| | $\mu$ | A | $\mu$ | A | $\mu$ | A | $\mu$ | A | $\mu$ | A |
| A. Bacteria | | | | | | | | | | |
| *Acinetobacter calcoaceticus* NRC 31015 | 0.54 | 0.73 | | | 0.58 | 0.81 | 0.35 | 0.58 | 0 | 0.008 |
| *Arthrobacter globiformis* NRC 32001 | 0.35 | 1.0 | | | 0.40 | 0.88 | 0.31 | 0.72 | 0 | 0.065 |
| *Arthrobacter luteus* NRC 21755 | 0.37 | 0.49 | | | 0.15 | 0.64 | 0.10 | 0.56 | 0 | 0.023 |
| *Arthrobacter* sp. NRC 32005 | 0.16 | 0.58 | | | 0.19 | 0.62 | 0.16 | 0.54 | 0 | 0.040 |
| *Bacillus* sp. RC607 | 0.66 | 1.1 | 0.58 | 1.3 | 0.60 | 1.3 | | | | |
| | 0.86 | 1.1 | | | 0.53 | 0.92 | 0.49 | 1.3 | | 0.22[b] |
| *Bacillus cereus* NRC 3045 | 0.72 | 1.3 | | | 0.51 | 1.3 | 0.07 | 0.95 | 0 | 0.039 |
| *Cytophaga johnsonii* NRC 39001 | 0.53 | 1.2 | | | 0.48 | 1.1 | 0.27 | 0.97 | 0 | 0.018 |
| *Enterobacter aerogenes* NRC 2926 | 0.58 | 0.56 | | | 0.83 | 0.52 | 0.54 | 0.75 | 0 | 0.089 |
| *Klebsiella aerogenes* | 0.42 | 0.41 | 0.44 | 0.46 | 0.51 | 0.59 | 0.39 | 0.85 | | |
| NCTC 418 | 0.39 | 0.41 | | | 0.63 | 0.66 | 0.45 | 0.88 | 0 | 0.095 |
| *Pseudomonas ambigua* | 0.72 | 1.0 | 0.54 | 0.91 | 0.24 | 0.95 | 0 | 0.030 | | |
| *Pseudomonas fluorescens*-like FR1 | 0.73 | 0.52 | 0.66 | 0.49 | 0.40 | 0.36 | 0.23[a] | 0.050 | | |
| *Pseudomonas fluorescens* NCIMB 11764 | 0.48 | 0.95 | 0.58 | 0.76 | 0.58 | 0.62 | 0.23[a] | 0.087 | | |
| *Pseudomonas fluorescens* NRC 2137 | 0.60 | 0.73 | | | 0.57 | 0.88 | 0 | 0.015 | 0 | 0.021 |
| *Pseudomonas fluorescens* NRC 2898 | 0.69 | 0.83 | | | 0.58 | 0.80 | 0.20 | 0.57 | 0 | 0.050 |
| *Pseudomonas putida* ATCC 33015 | 0.30 | 0.73 | 0.42 | 0.66 | 0.36 | 0.89 | 0.17 | 0.76 | 0 | 0.050 |
| *Pseudomonas putida* P.S. | 0.63 | 0.65 | 0.76 | 0.60 | 0.76 | 0.62 | 0.37 | 0.30 | | |
| *Pseudomonas putida* | 0.61 | 0.78 | 0.49 | 0.72 | 0.51 | 0.63 | 0.38 | 0.62 | | |
| PAW 340 | 0.49 | 0.86 | | | 0.81 | 0.74 | 0.58 | 0.68 | 0.063 | 0.052 |
| *Pseudomonas putida* NRC 2986 | 0.81 | 0.75 | | | 0.78 | 0.63 | 0 | 0.021 | 0 | 0.025 |
| *Pseudomonas putida* NRC 5019 | 0.54 | 0.63 | | | 0.63 | 0.55 | 0.60 | 0.50 | 0 | 0.042 |
| *Pseudomonas stutzeri* AG 259 | 0.33 | 1.3 | 0.48 | 0.13 | 0.45 | 1.2 | 0.37[a] | 0.11 | | |
| *Rhodopseudomonas capsulata* NRC 2199 | 0.34 | 0.44 | | | 0.17 | 0.70 | 0.14 | 0.50 | 0.074 | 0.11 |

Table 1 (continued)

| Organisms | $GeO_2$ Concentration (mg/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | 0.1 | | 0.5 | | 1.0 | | 2.0 | |
| | $\mu$ | A | $\mu$ | A | $\mu$ | A | $\mu$ | A | $\mu$ | A |
| B. Yeasts | | | | | | | | | | |
| *Aureobasidium pullulans* NRC 5673 | 0.26 | 1.2 | | | 0.24 | 0.90 | 0 | 0.050 | | |
| *Candida guilliermondii* NRC 5578 | 0.46 | 1.5 | 0.26 | 0.80 | 0.12[a] | 0.16 | 0 | 0.040 | | |
| *Candida shehatae* NRC 2886 | 0.17 | 0.68 | 0.10 | 0.68 | 0.10[a] | 0.15 | 0 | 0.042 | | |
| *Debaryomyces hansenii* NCYC 459 | 0.24 | 1.2 | 0.23 | 1.1 | 0.15 | 1.2 | 0 | 0.11 | | |
| *Pachysolen tannophilus* NRRLY2460 | 0.23 | 1.4 | 0.18 | 1.1 | 0.03 | 0.082 | 0 | 0.061 | | |
| *Pichia stipitis* NRC 2548 | 0.35 | 1.4 | 0.41 | 1.2 | 0.03 | 0.12 | 0 | 0.021 | | |
| *Rhodotorula mucilaginosa* NRC 211003 | 0.36 | 1.0 | 0.41 | 0.93 | 0.28 | 1.0 | 0 | 0.032 | | |
| *Rhodotorula rubra* NS-76-138 | 0.29 | 1.1 | 0.34 | 1.2 | 0.26 | 1.1 | 0 | 0.035 | | |
| *Saccharomyces cerevisiae* X2180-1B | 0.21 | 1.2 | 0.33 | 1.1 | 0 | 0.040 | 0 | 0.015 | | |
| *Saccharomyces cerevisiae* Y44 | 0.53 | 1.2 | 0.49 | 1.1 | 0.064 | 0.14 | 0 | 0.032 | | |
| *Saccharomyces uvarum* NRC 2417 | 0.43 | 1.2 | | | 0.16 | 0.88 | 0 | 0.042 | | |
| *Schwanniomyces alluvius* NRC 2509 | 0.38 | 0.95 | | | 0 | 0.18 | 0 | 0.057 | | |
| *Schwanniomyces castellii* NRC 2676 | 0.32 | 1.1 | | | 0.086 | 1.1 | 0 | 0.051 | | |

$\mu$ is the growth rate which is expressed as $h^{-1}$.
A is absorbance at 600 nm of the undiluted culture at 24 h.
[a] Growth was transient and was not sustained beyond 8 h.
[b] *Bacillus* sp. RC607 had an A at 48 h of 1.3 in the presence of 2 mg/ml $GeO_2$.

ml ($9.6 \times 10^{-2}$ M or 10 000 ppm) $GeO_2$ was prepared by dissolving an appropriate amount of the metal dioxide in a 0.25 M NaOH solution.

*Inocula preparation*

A loopful of bacteria from a nutrient agar slant was transferred to 5 ml of nutrient broth (Difco) supplemented with 0.5% (w/v) glucose. The culture was incubated in a loosely capped test tube (1.6 × 15 cm) which was rotated about its vertical axis as described by Schneider et al. [7], except the rotating axis was maintained at 60 degrees from horizontal. Cultures were grown at 28°C for 18 h. For yeast cultures, a loopful of cells was grown in 5 ml of medium containing 0.67% (w/v) yeast nitrogen base without amino acids (Difco) and 0.5% (w/v) glucose, at 28°C for 24 h.

*Growth response in the presence of $GeO_2$*

A sample of the inoculum culture was added to 5 ml of medium containing nutrient broth, 0.5% (w/v) glucose and 0.1, 0.5, 1 or 2 mg/ml $GeO_2$. For yeast cultures, the medium used contained 5 ml of 0.67% (w/v) yeast nitrogen base without amino acids, 0.5% (w/v) glucose and 0.1, 0.5 or 1 mg/ml $GeO_2$. The initial absorbance of the cultures at 600 nm ranged from 0.04 to 0.09. Control cultures were grown in the same manner except that $GeO_2$ was excluded. The cultures were grown in loosely capped test tubes and agitated at 28°C by rotation. Growth was monitored at various time intervals by direct measurement of the absorbance of the culture at 600 nm through the tubes using a Milton Roy Spectronic 20 spectrophotometer. The cultures were vortexed vigorously prior to measurement. Growth rate, ($\mu$), was determined by linear regression during early log phase. The growth yield was estimated as the absorbance (A) of the undiluted culture at 24 h. Growth was judged to have occurred when more than two doublings of the absorbance value immediately after inoculation were observed.

*Growth adaptation of bacteria to $GeO_2$*

The effect of prior exposure to $GeO_2$ on selected bacterial strains was investigated. Cells were initially grown as described earlier in the presence of 1 mg/ml $GeO_2$ for 24 h. A sample of the culture was used to inoculate the test medium containing 2 mg/ml $GeO_2$. Growth response was determined as above and compared to that without preincubation with $GeO_2$.

*Effect of culture pH and low incubation temperature on $GeO_2$ toxicity*

Bacterial strains were grown in the absence or the presence of 1 mg/ml $GeO_2$. Prior to inoculation, the pH of the medium was adjusted to 9 or 10 using sterile 1 N $HNO_3$. The effect of incubation temperature was investigated by incubating cultures at 10 or 28°C. The growth responses were determined as described above.

## RESULTS

*Growth response in the presence of $GeO_2$*

An initial screening of bacterial and yeast strains was performed to determine toxic concentrations of $GeO_2$. Table 1 shows the growth rate ($\mu$) and the growth yield (A at 24 h) of each strain in the presence of selected concentrations of $GeO_2$. In the screening experiments, the pH was not titrated back to 7 after $GeO_2$ addition. Therefore, the pH values of medium containing 0.5, 1 and 2 mg/ml $GeO_2$ were approximately 8, 9 and 10, respectively.

In bacteria, $GeO_2$ was not toxic when present at 0.1 mg/ml (Table 1). At 0.5 mg/ml, $GeO_2$ inhibited the growth rates ($\mu$) of several bacterial strains (*Arthrobacter luteus* NRC 21755, *P. ambigua, P. fluorescens* FRI and *Rhodopseudomonas capsulata* NRC 2199) by more than 40%. At 1 mg/ml, most of the bacteria exhibited both reduced growth rate and growth yield. The exceptions were *Arthrobacter* sp. NRC 32005, *Enterobacter aerogenes* NRC 2926, *Klebsiella aerogenes* NCTC 418 and *P. putida* NRC 5019, all of which could be classified as $GeO_2$-tolerant. However, none of these strains grew in the presence of 2 mg/ml $GeO_2$. Several other bacterial strains displayed slightly lower tolerance as shown by diminished growth rates but similar growth yields in the presence of 1 mg/ml $GeO_2$ (Table 1). *P. putida* ATCC 33015, the strain shown previously by Klapcinska and Chmielowski [5] to accumulate $GeO_2$ intracellularly, was among this group of bacteria.

Six sensitive bacterial strains did not grow at 1 mg/ml $GeO_2$ as evidenced by 24 h absorbance values which indicated less than one doubling of growth. All 6 strains were members of the genus *Pseudomonas*. Since the initial pH of the medium containing 1 mg/ml $GeO_2$ was approximately 9, the question arose as to whether the growth of these strains was inhibited by $GeO_2$, high pH, or both. On inoculating these sensitive strains in a liquid medium at pH 9 in the absence of $GeO_2$, all grew within 24 h (data not shown). Thus, the growth inhibition was most likely due to the presence of $GeO_2$.

The possibility that $GeO_2$ and high pH may act synergistically to inhibit microbial growth was not investigatged.

At 2 mg/ml $GeO_2$, only *Bacillus* sp. RC607 showed evidence of growth as judged by absorbance readings of 0.22 and 1.3 after 24 and 48 h, respectively. The medium containing 2 mg/ml $GeO_2$ had an initial pH of 10. Many bacterial strains may not be able to grow at this high pH, and the growth-inhibitory effect of Ge could not be definitively established. However, *Arthrobacter luteus, Arthrobacter* sp., *Bacillus* sp. RC607, *B. cereus* NRC 3045, *E. aerogenes* and *K. aerogenes* all grew in the absence of $GeO_2$ at pH 10 (data not shown). Therefore, the presence of high levels of $GeO_2$ in the medium was most likely to be responsible for inhibiting the growth of these strains.

Yeasts were generally more sensitive to the toxic effects of $GeO_2$ than bacteria. Reduced growth rates were evident with some of the strains at $GeO_2$ concentrations as low as 0.1 mg/ml (Table 1). The most sensitive strains were identified as *Candida guilliermondii* NRC 5578 and *C. shehatae* NRC 2886 on the basis of growth characteristics.

At 0.5 mg/ml $GeO_2$, all yeast strains exhibited reduced growth rates and/or growth yields. Based on growth yield inhibition, *Aureobasidium pullulans* NRC 5673, *Debaryomyces hansenii* NCYC 459, *Rhodotorula mucilaginosa* NRC 211003 and *R. rubra* NS-76-138 were found to be the most tolerant strains. *Schwanniomyces castellii* NRC 2676 exhibited very slow growth yet a very high absorbance value at 24 h. None of the yeasts tested grew in the presence of 1 mg/ml $GeO_2$. The high initial pH of the medium may be partially responsible for the growth inhibition of yeast cultures.

*Modulation of $GeO_2$ tolerance*

The ability of 4 bacterial strains to adapt to the presence of $GeO_2$ was investigated. The 4 strains tested were *Arthrobacter luteus* NRC 21755, *Bacillus* sp. RC607, *Enterobacter aerogenes* NRC 2926 and *Klebsiella aerogenes* NCTC 418. Since medium containing high concentrations of $GeO_2$ has a high pH, the strains chosen were those found to be alkalo-tolerant, i.e., capable of growth at pH 10. All grew poorly or not at all in the presence of 2 mg/ml $GeO_2$ (Table 1). However, inocula from cultures of these strains which were pre-grown for one day in the presence of 1 mg/ml $GeO_2$, grew at 2 mg/ml $GeO_2$ (data not shown). This suggests the existence of efficient adaptive mechanisms in these cells to resist the toxic effects of the metal.

The uptake of $GeO_2$ by some algal strains is highly pH-dependent [8]. Accumulation of $GeO_2$ by several algal cultures was significantly enhanced when the pH of the medium was shifted to higher values. Therefore, it was of interest to determine if tolerance to $GeO_2$ can also be modulated by a similar pH effect in bacteria. Two bacterial strains, *B. cereus* NRC 3045 and *P. fluorescens* NRC 2137, were used in this study. Both grew at pH 7 in a medium containing 1 mg/ml $GeO_2$. They could also grow at pH 10 and 9, respectively, in the absence but not in the presence of 1 mg/ml $GeO_2$. Pre-growth at high pH was carried out by growing *B. cereus* and *P. fluorescens* cultures at pH 10 and 9, respectively, for 24 h. On inoculating these cultures into fresh medium at pH 10 or 9, containing 1 mg/ml $GeO_2$, growth occurred readily in the *B. cereus* but not *P. fluorescens* culture (Table 2). These results clearly illustrate that modulation of $GeO_2$ tolerance by pH is strain-dependent.

The effect of cold temperature stress on $GeO_2$ toxicity was also investigated. *B. cereus* NRC 3045 and *P. fluorescens* NRC 2137 cultures were grown in the absence or the presence of 1 mg/ml $GeO_2$. The pH was adjusted to 7.0 with sterile 1 N $HNO_3$ and the tube cultures were incubated at 10 or 28°C with rotation. In the absence of $GeO_2$, both strains grew at 10 or 28°C as shown by the relatively high absorbance values obtained after 24 or 48 h incubation (Table 3). Both cultures also showed good growth at 28°C in the presence of the metal dioxide (Table 3). However, $GeO_2$ inhibited the growth of *B. cereus* cultures completely and that of *P. fluorescens* cultures partially at 10°C. These results suggest that temperature stress on the cell may enhance $GeO_2$ toxicity, and that this effect was strain-dependent.

Table 2

Effect of adaptation to high pH on $GeO_2$ toxicity at 28°C

| Bacteria | pH | pH adaptation[a] | $GeO_2$ (mg/ml) | A | |
|---|---|---|---|---|---|
| | | | | 22 h | 46 h |
| *Bacillus cereus* NRC 3045 | 7 | Yes | 0 | 1.2 | 1.3 |
| | | | 1.0 | 0.75 | 0.86 |
| | | No | 0 | 1.2 | 1.2 |
| | | | 1.0 | 0.70 | 0.95 |
| | 10 | Yes | 0 | 1.2 | 1.2 |
| | | | 1.0 | 0.59 | 1.3 |
| | | No | 0 | 1.3 | 1.4 |
| | | | 1.0 | 0.085 | 0.09 |
| *Pseudomonas fluorescens* NRC 2137 | 7 | Yes | 0 | 0.75 | 0.95 |
| | | | 1.0 | 0.76 | 0.78 |
| | | No | 0 | 0.75 | 0.85 |
| | | | 1.0 | 0.73 | 0.89 |
| | 9 | Yes | 0 | 0.85 | 1.0 |
| | | | 1.0 | 0.02 | 0.02 |
| | | No | 0 | 0.85 | 1.0 |
| | | | 1.0 | 0.01 | 0.01 |

[a] pH adapted cultures used inocula which were pre-grown at pH 10 or 9 for one day prior to the growth test.

Table 3

Effect of low temperature stress on $GeO_2$ toxicity

| Bacteria | Temperature (°C) | $GeO_2$ (mg/ml) | A | |
|---|---|---|---|---|
| | | | 24 h | 48 h |
| *Bacillus cereus* NRC 3045 | 10 | 0 | 0.54 | 1.2 |
| | | 1.0 | 0.12 | 0.11 |
| | 28 | 0 | 1.2 | 1.3 |
| | | 1.0 | 1.1 | 1.3 |
| *Pseudomonas fluorescens* NRC 2137 | 10 | 0 | 0.70 | 0.85 |
| | | 1.0 | 0.070 | 0.43 |
| | 28 | 0 | 0.90 | 1.0 |
| | | 1.0 | 0.72 | 0.85 |

## DISCUSSION

In general, the growth responses of the organisms were adversely affected as $GeO_2$ concentrations increased. The toxic effect was initially observed as a decrease in the growth rate. As the cultures continued to grow with reduced growth rates, some reached absorbance values after 24 h comparable to those of the same strains in the absence of $GeO_2$ while others did not. The toxicity of $GeO_2$ to the organisms may be a generalized adverse effect on either the growth rate, growth yield or both. Both of these parameters can be used to assess the relative tolerance of organisms to $GeO_2$. The precise cellular mechanism(s) responsible for $GeO_2$ toxicity is unknown. The toxic effects of Ge may be related to the extent of Ge accumulation. Cells which accumulate Ge may be the most sensitive strains while the more tolerant strains may possess mechanism(s) for Ge immobilization. The relationship between Ge-tolerance and its accumulation requires further investigation.

Ge binding has been reported in bacteria and algae [1,5,8]. However, the nature of the binding and accumulation mechanisms is not known. The ability of some bacteria to adapt quickly on preexposure to $GeO_2$ suggests the existence of immobilization or extrusion mechanisms. More research is necessary if organisms are to be used in Ge accumulation and recovery.

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