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Removal of Cr(VI) from ground water by *Saccharomyces cerevisiae*

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Abstract

Chromium can be removed from ground water by the unicellular yeast, *Saccharomyces cerevisiae*. Local ground water maintains chromium as CrO_4^{2-} because of bicarbonate buffering and pH and E_h conditions (8.2 and +343 mV, respectively). In laboratory studies, we used commercially available, nonpathogenic *S. cerevisiae* to remove hexavalent chromium [Cr(VI)] from ground water. The influence of parameters such as temperature, pH, and glucose concentration on Cr(VI) removal by yeast were also examined. *S. cerevisiae* removed Cr(VI) under aerobic and anaerobic conditions, with a slightly greater rate occurring under anaerobic conditions. Our kinetic studies reveal a reaction rate (V_{max}) of 0.227 mg h^{-1} (g dry wt biomass)$^{-1}$ and a Michaelis constant (Km) of 145 mg/l in natural ground water using mature *S. cerevisiae* cultures. We found a rapid (within 2 minutes) initial removal of Cr(VI) with freshly hydrated cells [55–67 mg h^{-1} (g dry wt biomass)$^{-1}$] followed by a much slower uptake [0.6–1.1 mg h^{-1} (g dry wt biomass)$^{-1}$] that diminished with time. A materials-balance for a batch reactor over 24 hours resulted in an overall shift in redox potential from +321 to +90 mV, an increase in the bicarbonate concentration (150–3400 mg/l) and a decrease in the Cr(VI) concentration in the effluent (1.9–0 mg/l).

Introduction

Metal-refining industries, mining operations, and manufacturing industries produce wastes that may contain heavy metals. Occasional leaching of the waste solids by rain percolation or surface water runoff has been a source of contamination of ground and surface water. The Lawrence Livermore National Laboratory (LLNL) site contains three areas where ground water is contaminated with volatile organic compounds (VOC) and Cr(VI). After VOC removal, the Cr(VI) concentration must not exceed 11 μg/l as specified in LLNL's National Pollutant Discharge Elimination System (NPDES) permit. The NPDES discharge limit is lower for Cr(VI) than the California Maximum Contaminant Level (100 μg/l).

Chromium is a common contaminant because of its wide-spread use in plating shops, tanneries, and aerospace facilities. Also, it has been found in the effluent of facilities with water towers and steam generators as well as in mine tailings. In situ ground water contaminated with chromium may have chromate ions in different ionic forms with total chromate concentration, pH, and E_h dictating the particular chromate species. Epidemiological data suggest that chromium compounds are carcinogenic in humans (International Agency for Research on Cancer 1973; Shi et al. 1991). Studies provide evidence that Cr(VI) salts of Na, K, and Ca are mutagenic (Venitt & Levy 1974).

Several species of bacteria, yeast, and algae are capable of accumulating metal ions extracellularly or internally to concentrations several orders of magnitude higher than the background concentration, and many bacteria reduce Cr(VI) to trivalent chromium [Cr(III)] (Wang & Shen 1995). *Pseudomonas* species have been studied and characterized for chromate reductase activity (Ishibashi et al. 1990). Although *P. aeruginosa* and *P. fluorescens* successfully removed Cr(VI) from ground water in studies conducted at LLNL (unpublished results), these organisms also taint the water with an unpleasant odor. In addition, *P. aeruginosa* is a human pathogen. For these reasons, we

studied the safer microorganism, *Saccharomyces cerevisiae* for its ability to bioreduce and remove Cr(VI) compounds from ground water.

S. cerevisiae is capable of accumulating Co^{2+} and Cd^{2+} (Norris & Kelly 1977); U (Strandberg et al. 1981); Cs, Sr, U (deRome & Gadd 1991); Cu(II) (Huang et al. 1990); and Cr^{2+}, CO^{2+}, and Cd^{2+} (Brady & Duncan 1994). Microorganisms respond to metals by several processes, including transport, biosorption to cell biomass, entrapment in extracellular capsules, precipitation, and oxidation-reduction reactions (Gadd & Griffiths 1978; Gadd 1990a, b). Bioaccumulation of metal cations has been demonstrated (Norris and Kelly 1977; Brady and Duncan 1994) by two processes: an initial rapid accumulation that is independent of metabolism and temperature, and a metabolically mediated process that internalizes the cation into the cell. Energy- dependent uptake of divalent cations by *S. cerevisiae* is well known (Fuhrmann & Rothstein 1968; de Rome & Gadd 1991) with influx being dependent on the electrochemical proton gradient across the plasma membrane (Borst-Pauwels 1981).

The purpose of our study was to investigate the use of a fermentative microorganism, *S. cerevisiae*, as an agent to remove Cr(VI) (1) in the laboratory using amended ground water and (2) on establishing chromate removal kinetics in batch cultures. In addition, we studied the effects on Cr(VI) removal rates for extracellular factors such as pH, temperature, and energy source concentration.

Materials and methods

For the laboratory studies, we used borosilicate glassware, which has low metal-cation binding properties. All glassware used for metals analyses was washed with detergent, rinsed, soaked in a 2–4% nitric acid bath, rinsed three times in ultra pure water (Millipore Milli-Q purification system), and autoclaved for sterility.

Ground water from LLNL was used for this study. It contains 32 µg/l Cr(VI), and is typical of the slightly alkaline (pH 8.2) and oxidizing conditions in ground water at LLNL. The ground water contained 280 mg/l $CaCO_3$ and total dissolved solids of 560 mg/l. The water was vacuum filtered through Millipore 0.45 µm filters.

Physical treatment of yeast cells

The organism used in this study was commercially produced, dehydrated *S. cerevisiae* (Fleischmann's, Oakland, California). In shake-flask experiments, *S. cerevisiae* was cultured in Sabouraud broth (SAB) containing dextrose (20.0 g/l), peptone (10.0 g/l) and 1 l of ultra pure water adjusted to pH 7 with 2 M HCl and autoclaved 25 min at 121°C. *S. cerevisiae* cultures were regenerated by weighing pelleted cells into 2% SAB in shaker flasks and incubated overnight at 30°C on a 220-rpm rotary shaker. Cells were harvested the next day by centrifuging in 200-ml Kimax centrifuge bottles for 10 min at 2500 rpm. The resulting cell pellet was then suspended in filtered ground water and centrifuged again for 10 min at 2500 rpm. The water was decanted off the pellet, and cells were resuspended in sterile, filtered ground water. Final cell density was about 8 g dry wt biomass/l.

In the reactor studies, the dehydrated yeast pellets were weighed and added directly to the test vessels, to give an initial concentration of about 8 g/l. Dried cell weights were obtained by filtering a known volume of the culture through a tared, 0.45-µm membrane filter; rinsing the resulting pellet; and drying the pellet at 80°C for 24 h.

For the reactor tests, the pH was monitored with an Ingold OPM 131 electrode (New Brunswick Sci. Co., Edison, New Jersey). For experiments with live cells, the pH was maintained at 6.5 by neutralizing the acid produced with 4 N NaOH. A strong alkali was chosen to limit dilution; cells in localized areas in the reactor may have died because of the strong base addition.

Cells were killed by three different methods: (1) autoclaving at 120°C and 2 ATM for 35 min, (2) irradiating with Cs^{137} for 90 min, or (3) adding mycostatin to the culture. Because autoclaving was the most reliable method of killing yeast cells, it was used for all subsequent experiments. Triplicate plating on SAB agar plates was used to verify that dead cells were nonviable.

Analytical methods

Measurements of redox potential were used to determine the oxidizing or reducing capability of the reactor fluid. Quinhydrone (Sigma Chemical Co., St. Louis, Missouri) was used to calibrate the E_h probe (Orion Ionanalyzer combination redox probe). The dissolved oxygen was measured by an oxygen probe (Ingold, polarographic). The redox potential readings

were reported relative to the normal hydrogen electrode, corresponding to the filling solution used and the temperature of the solution measured.

Glucose was provided as the carbon and energy source. Ground water was amended with 2 mg/l $K_2Cr_2O_7$ (Sigma Chemical Co. St. Louis, Missouri) unless otherwise noted. In the pH and E_h conditions of our ground water, $K_2Cr_2O_7$will dissociate to $Cr_2O_7^{2-}$, then further dissociate to CrO_4^{2-}. To distinguish the biotic from abiotic Cr(VI) removal, tests were run with chemical control (CC) series, dead-cell (DC) control series, and live-cell (LC) test series.

The Cr(VI) samples were collected in 25-ml aliquots in acid-washed, screw cap, 50-ml test tubes; they were analyzed spectrophotometrically using modified U. S. Environmental Protection Agency (EPA) methods (Standard Methods, 17th ed. 3500-Cr D. Colormetric method). Measurements were performed using 5-cm path-length cuvettes with a Shimadzu spectrophotometer UV160U at 540 nm absorbency under yellow room lighting for light-sensitive reagents.

Total chromium was analyzed with a Perkin-Elmer 5100 series Atomic Absorption Spectrophotometer (AAS) equipped with a 4-in. single-slot flame head. The AAS was outfitted with a Transverse High Temperature Graphite Furnace and AS–71 autosampler. A Perkin-Elmer chromium lamp was used as the energy source for both flame and furnace analyses. Biomass was collected on 0.45-μm Millipore filters and digested in a solution of 50:50 nitric and hydrochloric acid 24–48 h, depending on the digestion progress.

Determination of parameters affecting kinetics of process

Effect of pH on chromate removal

Ground water pH was adjusted with 2 N HCl to seven different pH levels (8.2 to 2.0) in 250-ml Erlenmeyer flasks. Each flask initially contained 1.5 g dry wt biomass/l, 50 mM glucose, and 2 mg/l Cr(VI), and was incubated for 24 h at 20°C. A duplicate set of flasks was inoculated with 1.5 g dry wt biomass/l of dead yeast and handled in the same manner. The cell suspensions were filtered through 0.45-μm membrane filters, and replicate aliquots of the supernatant were analyzed for Cr(VI).

Effects of temperature on chromate removal

Flasks containing filtered ground water and 25 mM glucose were inoculated with 8 g dry wt/l activated dry yeast. Ten different temperatures were tested, ranging from 15° to 45°C (15, 20, 22, 25, 27, 30, 32, 35, 40, and 45°C). Replicate sets of inoculated and uninoculated flasks were placed in incubators and allowed to come to temperature; then, 2 mg/l Cr(VI) was added to each flask. After a 2-h incubation period, a 10-ml aliquot from each flask was filtered through a preweighed 0.45-μm filter. The pellets were rinsed, dried, and weighed for biomass, and the filtered supernatants were analyzed for Cr(VI).

Effects of glucose concentration on chromate removal

In batch tests, we evaluated the effects of 50, 100, and 500 mM glucose concentration on Cr(VI) removal from ground water (pH 8.1). Using live yeast cells, the experiments were started with 8.25 g dry wt biomass/l and continued for 340 min. The initial concentration of Cr(VI) was 129 mg/l, and the incubation temperature was 30°C. We measured in replicate the Cr(VI) removal and biomass in the media intermittently over the 6-h period.

Kinetic studies

Kinetic studies on the interaction between Cr(VI) and *S. cerevisiae* were performed under mixed conditions on a rotary shaker (New Brunswick Sci. Co., 120 rpm). Chromate removal experiments were performed in filtered ground water (pH 8.1) containing Cr(VI) at concentrations ranging from 0.1–50 mg/l. The inoculum was 5 g dry wt biomass/l *S. cerevisiae*, and the incubations were performed at room temperature (21°C) with 25 mM glucose. We used CC and DC controls. Periodically, samples were removed from the suspension and filtered immediately through 0.45-μm membrane filters to remove the cells. This process was performed after 0, 0.5, 2, 4.5, 9, 12, 19, 24 and 36 h of mixing. Filtrates were analyzed for residual Cr(VI) concentration.

Reactor materials balance

An analysis of the feed and outflow liquid, gas, and solid streams was conducted for three reactor test sets: LC, CC and DC controls. For each test set, the ground water was amended with 2 mg/l (41.6 mM) Cr(VI). The reactor used in this study was a modified New Brunswick BiofloIII attached to a Neslab chiller and an E_h monitoring system that included a Tektronix AMZ amplifier for increasing the mV signal. Data were collected on a Macintosh SX computer using National Instrument LabView software. The reactor was allowed to come

Table 1. Cr(VI) removal by live *S. cerevisiae* biomass at several pH values.

Initial pH	Final pH	Initial Cr(VI) (mg/l)	Final Cr(VI) (mg/l)	Cr(VI) removal (%)
2.0	2.0	2.11	0.77	61
4.0	3.1	1.91	0.38	81
5.0	3.8	1.91	0.35	82
6.1	3.9	1.96	0.21	89
6.5	4.0	1.84	0.00	100
7.0	4.4	1.85	0.00	100
8.2	4.9	1.96	0.67	67

S. cerevisiae concentration = 1.5 g/l, glucose concentration = 50 mM, Cr(VI) concentration = 2 mg/l in ground water for a 24-h exposure period. The pH was adjusted with 2 N HCl, and the ambient test temperature was 20°C.

Table 2. Cr(VI) removal by dead *S. cerevisiae* biomass at several pH values.

Initial pH	Final pH	Initial Cr(VI) (mg/l)	Final Cr(VI) (mg/l)	Cr(VI) removal (%)
2.1	2.0	2.11	0.60	70
4.0	4.1	1.91	1.35	32
6.0	5.9	1.96	1.61	20
8.0	7.8	1.96	1.97	0

Initial *S. cerevisiae* concentration = 1.5 g/l, glucose concentration = 50 mM, autoclaved for 35 min at 121°C; Cr(VI) concentration = 2 mg/l in ground water for 24-h exposure period. The pH was adjusted with 2 N HCl, and the ambient test temperature was 20°C.

to steady state prior to inoculation; the testing period was 24 h, after which pH, E_h, and dissolved oxygen were measured.

At the end of each experiment, the reactor contents were divided into vapor, aqueous, and solid phases. The vapor phase from the reactor was collected in humidified Tedlar bags, and sent with air blanks and standards to a certified laboratory (Air Toxics Inc., Sacramento, California) for vapor analysis (gas chromatography and mass spectroscopy to identify all total ion chromatogram peaks). The culture aqueous phase was filtered through a Millipore membrane filter of 0.45-μM cellulose acetate. The filtrate was collected in Erlenmeyer vacuum flasks and sent to a certified laboratory (California Laboratory Services, Rancho Cordova, California) for analyses of general minerals and anions. The solid phase was the residual cell material remaining on the filter paper after aqueous phase collection. The cell mass was washed with analytical grade water and analyzed for total chromium.

Results and discussion

Effect of pH on Cr(VI) removal

We observed that the removal of Cr(VI) under varying pH conditions was different for live *S. cerevisiae* than for dead *S. cerevisiae*. The optimum pH for viable *S. cerevisiae* for removal of Cr(VI) from solution was near neutrality. Between pH values of 6.5 and 7, 100% Cr(VI) was removed from the amended ground water sample (Table 1). The LC and DC cultures showed opposite results over a pH range of 2–8. In more acidic conditions, the Cr(VI) removed by live *S. cerevisiae* became less efficient whereas at the natural pH of ground water (8.2), 67% of the Cr(VI) was removed. In contrast, dead *S. cerevisiae* in ground water with a pH of 8.0 did not remove Cr(VI) from solution, however at a pH value of 2.1, 70% of the Cr(VI) was removed from the filtrate (Table 2). In the DC controls, we observed an inverse relationship of pH and Cr(VI) removal between pH 2 and 8. The proportion of absorption versus abiotic effects was not determined. Processes that may remove chromate from solution include absorption of Cr(III) by biomass, abiotic reduction of Cr(VI) at low pH, or enzymatic reduction of Cr(VI) by *S. cerevisiae. S. cerevisiae* grow actively at pH values lower than those optimal for most bacteria.

Effect of temperature on Cr(VI) removal by S. cerevisiae

The optimum Cr(VI) removal rate occurred with incubation temperatures 25°–35°C. The shake flasks that were maintained at 15°C resulted in a Cr(VI) removal rate of 0.037 mg hr^{-1} (g dry wt biomass)$^{-1}$ compared to 0.094 mg h^{-1} (g dry wt biomass)$^{-1}$ from test flasks maintained at 25°C. The maximum rate of 0.107 mg h^{-1} (g dry wt biomass)$^{-1}$was obtained from the test flasks held at 35°C. Other authors have cited increased metal uptake with elevated temperatures. Brady and Duncan (1994) studied the accumulation of copper with *S. cerevisiae,* and determined that the optimum temperature for maximum metal removal was 25°–30°C. Strandberg et al. (1981) determined that the rate of uranium uptake increased with the temperature range 20°–50°C .

Table 3. Cr(VI) removal rates with 50, 100, and 500 mM glucose in shake flasks during a 6 h incubation period.

Glucose (mM)	Time (min)	Removal of Cr(VI) (mg/h/g dry wt biomass)
50	2	54.94
	30	0.63
	60	2.76
	120	1.43
	160	0.97
	340	0.60
100	2	54.81
	30	7.36
	60	4.02
	120	2.12
	160	1.84
	340	1.12
500	2	64.19
	30	4.97
	60	2.41
	120	1.65
	160	1.44
	340	0.83

Experimental conditions: 30° C; initial Cr(VI) concentration of 129 mg/l; 8 g/l dry wt of *S. cerevisiae* inoculum; initial pH 8.1 in ground water in culture vessels. Removal of Cr(VI) mg/h/g dry wt biomass was calculated by dividing Cr(VI) mg removed from the supernatant by the g dry wt biomass. The results are the average of replicate samples.

Effect of glucose concentration on the removal of Cr(VI)

A glucose concentration of 100 mM in yeast cultures resulted in the best Cr(VI) removal rate from samples taken during 340 min of cell contact time (Table 3). In the absence of yeast, glucose concentrations up to 500 mM in the Cr(VI) amended ground water resulted in no Cr(VI) removal. Cr(VI) removal followed two-step kinetics. The live yeast cells removed approximately 55 mg Cr(VI) h^{-1} (g dry wt biomass)$^{-1}$ in the first two minutes (Table 3). After the initial uptake, the subsequent Cr(VI) removal required glucose and occurred at a much slower rate. The initial accumulation was probably caused by biosorption of Cr(VI) cations to the biomass, possibly the cell wall. The second, slower phase may have resulted from metal internalization and enzyme dependence.

Rate of chromate removal

In Figure 1, we show a double reciprocal plot of rates of chromate removal attained by *S. cerevisiae* during 0-order growth. This plot shows the rate of chromate removal by yeast cells as a function of initial chromate concentration in the flask. *S. cerevisiae* removed Cr(VI) in the form of chromate at concentrations of 0.1–50 mg/l. The experiments were performed under completely mixed, anoxic conditions, using glucose (100 mM) as a carbon source. The rate of chromate removal to concentrations below the detection limit was 0.227 mg Cr(VI) h^{-1} (g dry wt biomass)$^{-1}$ and Km at 145 mg/l chromate. The high K value indicates a lack of strong affinity for Cr(VI). Our results agree with those of Mehlhorn et al. (1995) at a rate of Cr(VI) reduction of 0.2 mg Cr(VI) h^{-1} (g dry wt biomass)$^{-1}$ obtained by using an electron spin resonance (ESR) technique (Mehlhorn et al. 1993). Mehlhorn et al. (1995) determined by analysis that the Cr(VI) was being reduced. They analyzed a transient paramagnetic Cr(V)-complex that arises from the univalent reduction of Cr(VI) by thioglycerol and complexing Cr(V) with glycerol and thioglycerol.

We compared the *S. cerevisiae* Cr(VI) removal rate to that of *P. fluorescens* (isolated from LLNL soils contaminated with BTEX). The removal rate of *P. fluorescens* used in our test conditions, using ground water and environmental temperatures, was significantly slower than that of *S. cerevisiae*. For *S. cerevisiae*, the rate of removal was 0.227 mg h^{-1} (g dry wt biomass)$^{-1}$. For *P. fluorescens* it was 0.47 μg h^{-1} (g dry wt biomass)$^{-1}$, although other biotypes of *P. fluorescens* may show different Cr(VI) removal rates. Bacterial kinetic studies revealed that chromium removal rates vary with experimental conditions. A first-order constant of chromium removal of 0.1518 h^{-1} for an initial concentration of 1,000 mg/l was determined in a study using a consortium of sulfate-reducing bacteria (Fude et al. 1994). A V_{max} of Cr(VI) reduction of 4.01 mg h^{-1} (g dry wt biomass)$^{-1}$ was determined in a study using *P. aeruginosa* PA01 (Apel & Turick 1992). Several authors concluded that the rate of Cr(VI) reduction depended on the concentration of Cr(VI) in the culture vessel (Fude et al. 1994; Shen & Wang 1994).

Initially, *S. cerevisiae* removes Cr(VI) from the solution by absorption. Our studies suggest that the initial rapid Cr(VI) removal is followed by a significantly slower metabolically mediated Cr(VI) removal (Table 3). A similar effect was noted by Brady & Duncan (1994) in Cu^{2+} bioaccumulation experiments. They

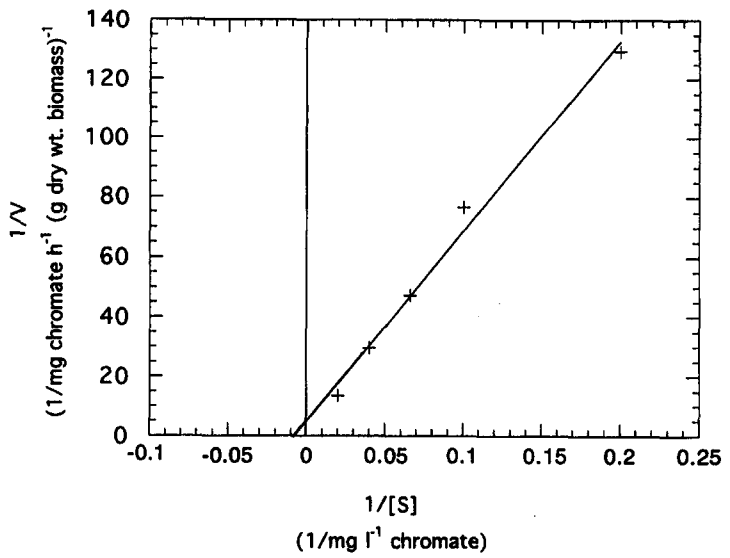

Figure 1. Double reciprocal plot of Cr(VI) reduction by *S. cerevisiae*: V_{max} = 0.227 mg h^{-1} (g dry wt biomass)$^{-1}$, K_m = 145 mg/l. Samples were removed, filtered and analyzed for Cr(VI) after 0, 0.5, 2, 4.5, 9, 12, 19, 24 and 36 h of cell contact.

determined that the accumulation by yeast was dependent on the ratio of external, free metal, ion concentration to the available biomass.

In our bioreactor studies, we found that 45–52% of the Cr(VI) was removed from solution by the dead biomass. Mehlhorn et al. (1995) has shown that a regime of freeze-thawing the yeast cells greatly enhanced their ability for Cr(VI) reduction. Both processes, autoclaving and freeze-thawing, break cell membranes and may increase additional nonspecific binding of the chromium ion.

Strandberg et al. (1981) found a rapid uptake of uranium on the cell surface of *S. cerevisiae* and, after a 24-h exposure, the uranium was also deposited within the cells. These authors suggested that the kinetics of the biosorption process demonstrated the transport of the metallic ions to the cellular surface and eventually through the cell wall. Additionally, the authors found that living and nonliving cells of *S. cerevisiae* exhibit similar tendencies of binding uranium to the outside and inside of the cells. Volesky (1990) suggested that the overall kinetics is complicated by the different modes of biosorbent metal deposition.

Results of reactor experiments

Effects of aerobic versus anaerobic reactor condition on Cr(VI) removal

A series of reactor tests were conducted to measure the effect of air on the cell's ability to remove Cr(VI) from solution. In one test, air was introduced at 1.7 l/min, the maximum allowed by the reactor. The rate of Cr(VI) reduction/accumulation over time in the aerated test was compared to a reactor test in which no additional oxygen was provided. Both tests were conducted at the ground water temperature (19°C). The removal of Cr(VI) in the oxygen-limited reactor was approximately 10–20% greater than in the reactor provided with continuous filtered air (Fig. 2). The initial pH was 8.4 in the aerobic culture. After 2 h, the pH declined to 6.3; and after 24-h, the pH was 5.4. The culture grown without the addition of air also began with a pH of 8.4. After the 24-h test, the pH was 4.6.

Materials balance of a batch reactor

Although many chemical reactions occur in the reactor, only the elemental ratios of chromium and carbon compounds change greatly in the effluent. These byproducts depend on the level of chromium reduced, the heavy-metal concentration in the feed, and reactor pH and E_h, provided that optimal microbial conditions exist within the reactor. Microorganisms consume organic compounds, such as sugar, for growth and ener-

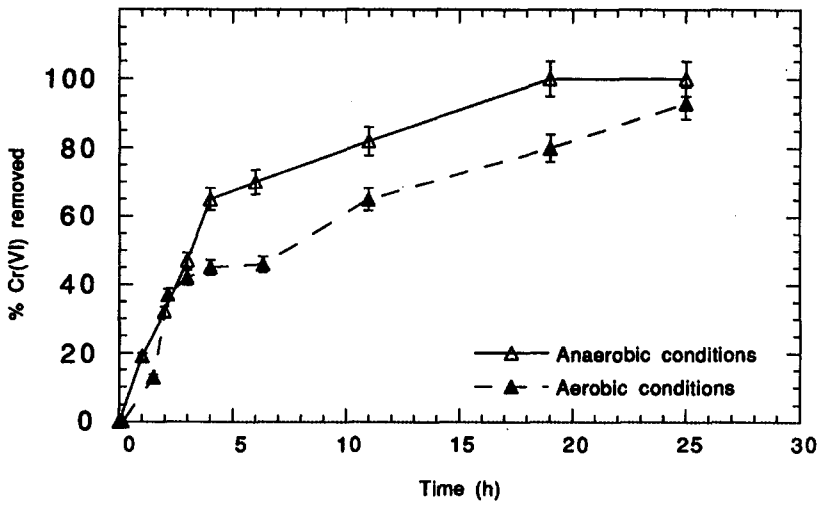

Figure 2. The effect of air on the cell's ability to accumulate Cr(VI). Two 24-h reactor test were conducted with an initial reactor composition of 10 g/l biomass, 100 mM glucose, and 2 mg/l Cr(VI). The aerated culture was initially saturated with oxygen, and throughout the run the oxygen level was controlled at 1.7/l/min with sterile filtered air at 19°C. The initial pH and E_h were 8.42 and +312 mV, respectively.

gy; a byproduct of this metabolism is the production of electrons. These electrons are used in the reduction of chromate to energetically stable chromium hydroxide [$Cr(OH)_3$]; its solubility is 6.7 x 10^{-31}mg/l). The overall reaction in an anaerobic, acidic environment, with glucose provided as the carbon source, may be represented by:

$$8H^+ + 2H_2O + (CH_2O)_6 + 4Cr_2O_7^{2-} \rightarrow 6CO_2 + 8Cr(OH)_3$$

In our material balance study, we examined both Cr(VI) and total chromium. Although we did not look at specific oxidation states, we assumed that the chromium existed in two oxidation states in our study: Cr(VI) and Cr(III). The CC culture Cr(VI) concentration did not change over the 24-h test period (Table 4). The CC culture total chromium concentrations were about 2 mg/l throughout the test, and chromium in the reactor was in the hexavalent state. In the second reactor test before the addition of the DC biomass, the Cr(VI) concentration was 2.10 mg/l. The concentration dropped to 1.0 mg/l immediately after the addition of the DC culture. After the 24-h incubation period, the Cr(VI) concentration dropped to 0.58 mg/l. Total chromium concentration dropped to 1.1 mg/l immediately after DC biomass was added; after 24 h, the concentration was 0.94 mg/l. In the DC culture effluent, 55% of the total chromium was removed, most likely by biosorption. Strandberg et al. (1981) found an increased rate of uranium uptake by dead *S. cerevisiae* biomass and suggested that it resulted from surface-associated biosorption. According to Wang and Shen (1995) yeast extract or nutrient broth alone may reduce Cr(VI) by organic compounds with sulfhydryl groups, especially in the absence of oxygen.

Cr(VI) in the LC culture was completely removed; however, 0.61 mg/l of a chromium species other than Cr(VI) remained in solution (Table 4). Possible explanations for chromium in the effluent are (1) depuration of the reduced metal from surface binding sites, (2) cell lysis, or (3) Cr(VI) reduction by extracellular enzymes. Another explanation is elution with sodium carbonates and bicarbonates. In studies conducted by de Rome and Gadd (1991), a 1-M solution of sodium carbonate/sodium bicarbonate was used to elute up to 80% cesium and strontium from *S. cerevisiae* biomass. Possibly, the high concentration of bicarbonates in our ground water eluted the reduced chromium from the biomass in the reactor. Additional research is needed to evaluate extra- and intracellular chromium oxidative states and fates.

The pH in the CC cultures was relatively constant, ranging from 8.2 to 8.3 without any need for adjustment. The reactor solution in the presence of LC culture has an initial pH of 8.1, but potentially may decline to 2 or 3 due to the fermentation of glucose, which produces organic acids such as lactic acid. Therefore, for the LC culture, we controlled the reactor pH to 6.5 with 4 M sodium hydroxide. This decision to adjust the pH to 6.5 was based on the minimum pH values

Table 4. Materials balance of aqueous influent and effluent, gaseous effluent and solid effluent in a 24 h bioreactor study.

Aqueous analyte (mg/l)	Influent	Effluent	Gaseous analyte	Effluent	Solid analyte	Effluent
Chemical Control						
Hexavalent chromium	2.08	2.10	Ethanol (ppmv)	1.5	Biomass $(CH_2O)_6$(mg/ml)	0.00
Total chromium	2.1	2.3	Carbon dioxide (%)	0.05	Total chromium	0.00
Bicarbonate	270	260				
Dissolved oxygen (%)	99	108				
E_h(mV)	310	307				
Lactate (mM/l)	<0.2	<0.2				
pH (units)	8.2	8.3				
Total dissolved solids	5000	4900				
Dead Cell Control						
Hexavalent chromium	2.1	0.58	Ethanol (ppmv)	14	Biomass $(CH_2O)_6$(mg/ml)	1.13
Total chromium	2.1	0.94	Carbon dioxide (%)	1.7	Total chromium	0.68
Bicarbonate	150	73				
Dissolved oxygen (%)	100	0				
E_h(mV)	345	336				
Lactate (mM/l)	<0.2	4.5				
pH (units)	8.3	6.4				
Total dissolved solids	5200	5000				
Live Cell Test						
Hexavalent chromium	1.87	0.00	Ethanol (ppmv)	300	Biomass $(CH_2O)_6$(mg/ml)	4.65
Total chromium	2.03	0.61	Carbon dioxide (%)	27	Total chromium	1.28
Bicarbonate	150	3400				
Dissolved oxygen (%)	99	0				
E_h(mV)	321	90				
Lactate	<0.2	<0.2				
pH (units)	8.1	6.5				
Total dissolved solids	5700	7100				

In each reactor study, ground water was used, Cr(VI) was added (2.0 mg/l), and glucose (50 mM/l) was added as a food source for the yeast. The reactor was maintained at 24° C at about 1 atm. The dead cell control and the live cell test were pH adjusted with 4 N NaOH. No oxygen was provided during these tests.

permitted for ground water surface discharge. The pH value of 6.5 was also chosen because yeast cells grow best at neutral pH values, and the optimum Cr(VI) removal in our studies occurs when the culture is maintained at neutral pH values. The pH in the DC cultures was about 8.3 at 0 h, and dropped to 6.4 by 24-h. The decrease was caused by bacterial contamination that occurred after about 15-h. Bacterial contamination was confirmed by E_h, pH, and dissolved oxygen measurements, and microscopic examination. We cultured these bacteria and spiked the culture with Cr(VI). The contaminating bacterial culture did not reduce Cr(VI) to Cr(III) or remove Cr(VI) by biosorption.

Redox potential

The redox potential (E_h) is determined by electron activity; matter in solution may either absorb or release electrons. The E_h will increase with the addition of dichromate, a strong oxidizer, to ground water. Fude et al. (1994) found E_h increases by approximately 150 mV with increasing Cr(VI) concentration up to 1,000 mg/l. In this study, local unamended ground water had a pH of 8.2 and an E_h of +343 mV at 20°C, a substantial oxidizing potential. A study of total chromium and Cr(VI) in ground water from 39 LLNL monitor wells was conducted by McNab and Narasimhan (1994). Data from these analyses suggested that the average contribution to Cr(VI) from total chromium was virtually 100% due to the pH and E_h conditions of the ground water.

Our data from LC inoculated reaction vessels suggested that reducing potential increased after approximately 5 h of acclimatization (Fig. 3). Reducing conditions developed only in the presence of viable cells.

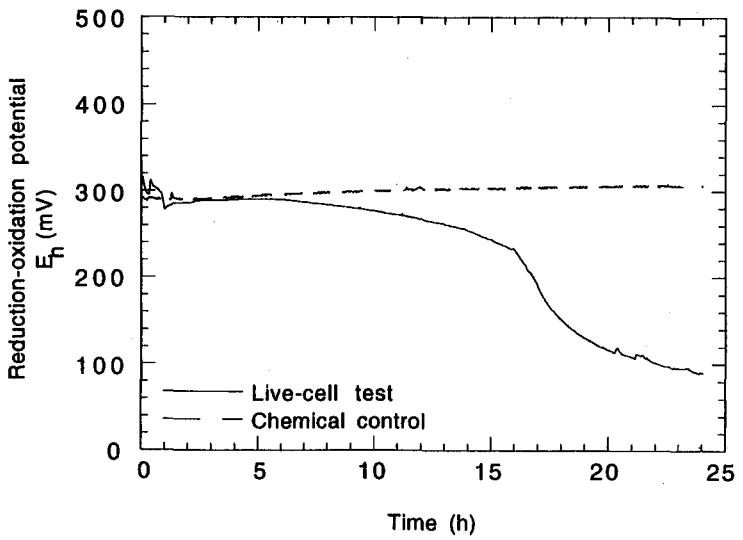

Figure 3. Redox measurements were electronically recorded in the reactor at one-minute intervals during three 24-h tests. The tests included a chemical control, a killed-cell, and the *S. cerevisiae* test series. Local ground water had a pH of 8.2 and an E_h of +343 at 20°C. The redox potential readings are reported relative to the normal hydrogen electrode corresponding to the filling solution used and the temperature of the solution measured.

The chemical control redox potential maintained E_h at a relatively stable +286 to +307 mV through the 24-h test period. The heat-killed cells increased E_h from +345 to about +420 mV. The DC culture E_h stabilized at values greater than the CC culture. The dead-cell biomass may have become an electron donor, thereby increasing the redox potential. Most Cr(VI) removal from the DC solution was likely due to biosorption and not reduction. However, 30% of the total chromium remaining was in an oxidation state other than Cr(VI), presumed to be Cr(III). In the LC culture, E_h began at +321 mV and decreased to +90 mV during the 24-h test period. The pH was not allowed to decrease below 6.5, and most likely, the addition of NaOH decreased the ability of the LC culture to reduce Cr(VI). Viable sulfate-reducing bacteria cultures developed reducing conditions (between +100 to +200) in experiments conducted by Fude et al. (1994). A solution with a negative E_h may be capable of reducing Cr(VI) to Cr(III); however, the best E_h range has not yet been established and does not always enhance Cr(VI) reduction. For example, *E. coli* ATCC 33456 in aerobic and anaerobic cultures drastically lowered the redox potential but did not significantly enhance Cr(VI) reduction (Shen and Wang, 1994).

Ground water minerals

The initial ground water bicarbonate ion concentration of the DC reactor was 150 mg/l (Table 4). After 24 h, the bicarbonate concentration decreased to 73 mg/l. In contrast, the LC bicarbonate concentration increased to 3400 mg/l. Equilibrium between HCO_3^- and CO_3^{2-} is influenced by cellular CO_2 produced during the metabolism of glucose; CO_2 dissolves in water as carbonic acid. The formation of carbonic acid (a very weak acid) and its salts is strongly influenced by the prevailing pH. The carbonate-bicarbonate equilibria in the bioreactor is an incidental consequence of metabolic processes that affect pH. The pH in most media is controlled by a combination of dissolved gases (bicarbonate buffer system) and products of metabolism by the cell (such as lactic acid). As the pH decreases, the equilibrium shifts toward bicarbonate production.

Specific conductance values changed significantly among the various groups. Specific conductance, or the ability of a solution to conduct a current, depends on the presence of ions, their total concentration, mobility, valence, and relative concentration. The specific conductance value of the CC culture was about 850 μmho/cm (25°C), typical for ground water containing chromate. The specific conductances of the DC culture were 980 and 960 μmho/cm at 0 and 24 h, respectively. This increased conductivity may be due to lysed cells. For the LC culture, the specific conductance values

were 840 and 5700 μmho/cm at 0 and 24 h, respectively. The increase in the specific conductance in the LC culture indicates an increase in electrolyte production.

Summary

We investigated the use of *S. cerevisiae* as an agent for reducing Cr(VI) to less toxic chromium oxidative states. To determine the optimum environmental parameters for *S. cerevisiae* metal removal, we evaluated such factors as pH, dissolved oxygen, temperature, and nutrient levels. In bioreactor studies, the chromium removal rate was slightly faster under anaerobic than aerobic conditions. We obtained an overall mass balance by analyzing the feed and outflow liquid, gas, and solids in a stirred-tank reactor. A control test, conducted in the absence of microorganisms, resulted in 100% recovery of the Cr(VI) in the reactor liquid effluent. The removal of Cr(VI) contamination from ground water with *S. cerevisiae* occurs at the moderate rate of 0.227 mg h^{-1} (g dry wt biomass)$^{-1}$.

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